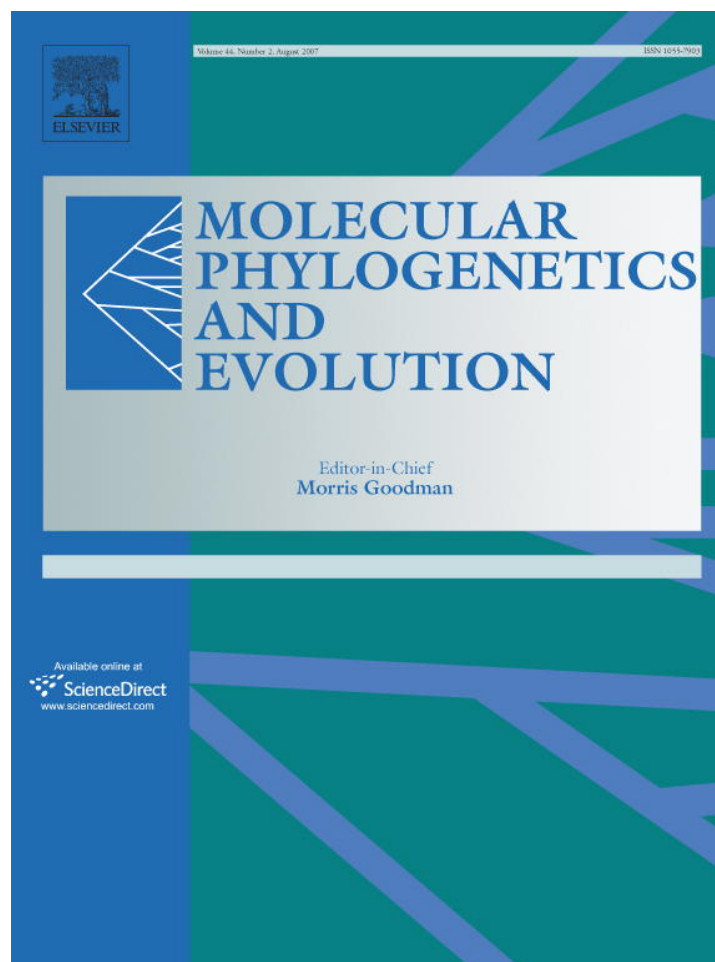


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A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification

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We dedicate this paper to Charles Jeffrey on the occasion of his 72nd birthday.

Abstract

Cucurbitaceae contain c. 800 species in 130 genera and are among the economically most important families of plants. We inferred their phylogeny based on chloroplast DNA sequences from two genes, one intron, and two spacers (*rbcL*, *matK*, *trnL*, *trnL-trnF*, *rpl20-rps12*) obtained for 171 species in 123 genera. Molecular data weakly support the traditional subfamilies Cucurbitoideae (111 genera) and Nhandioboideae (19 genera, 60 species), and recover most of the eleven tribes, but almost none of the subtribes. *Indofevillea khasiana* is sister to all other Cucurbitoideae, and the genera of Joliffieae plus a few Trichosantheae form a grade near the base of Cucurbitoideae. A newly discovered large clade consists of the ancestrally Asian genera *Nothoalsomitra*, *Luffa*, *Gymnopetalum*, *Hodgsonia*, *Trichosanthes*, and the New World tribe Sicyeae. Genera that are poly- or paraphyletic include *Ampelosicyos*, *Cucumis*, *Ibervillea*, *Neoachmandra*, *Psiguria*, *Trichosanthes*, and *Xerosicyos*. Flower characters, especially number of free styles, fusion of filaments and/or anthers, tendril type, and pollen size, exine, and aperture number correlate well with the chloroplast phylogeny, while petal and fruit characters as well as karyotype exhibit much evolutionary flexibility.

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Keywords: Cucurbitaceae; *matK*; Phylogenetics; *rbcL*; *rpl20-rps12* intergenic spacer; *trnL* intron; *trnL-trnF* intergenic spacer

1. Introduction

Cucurbitaceae, with 800 species in currently 130 genera (Jeffrey, 2005; De Wilde and Duyfjes, 2006a,b,d), are among the economically most important plant families. Cultivars developed by breeders, especially of pumpkin (*Cucurbita pepo*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), and water melon (*Citrullus lanatus*), are the basis for multi-million dollar industries, and the commercial role of derivatives from medicinal species is increasing rapidly. In spite of the family's economic importance, it has not yet been studied using quantitative methods of data analysis on molecular or morphological characters. Of the 130 genera, some 50 contain a single species, which

illustrates the difficulties of deducing Cucurbitaceae relationships from morphology and also reflects the economic importance of the family, with many names dating back to Medieval or even Greek and Roman medical and horticultural treatises. The most important diagnostic characters for the genera and tribes of Cucurbitaceae come from androecium and gynoecium morphology, and type of tendril branching (Cogniaux, 1881, 1916; Cogniaux and Harms, 1924; Müller and Pax, 1889; Jeffrey, 1962a, 1967, 1980, 1990a,b, 2005; for a history of cucurbit classification see Jeffrey, 1967). Since the 1960s, pollen structure has been used as an additional criterion to diagnose certain tribes (Marticorena, 1963; Jeffrey, 1964, 1990a,b). Seed coat characters were added more recently (Jeffrey, 1990b, 2005). The testa of Cucurbitaceae is formed by the outer integument and consists of a lignified epidermis, a hypodermis of one or many layers of sclerotic cells, and an inner one-layered

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protective cover that in mature seeds is heavily lignified (Singh and Dathan, 2001).

The family Cucurbitaceae has traditionally been divided into two subfamilies, the Nhandioboideae (often still referred to by the younger name Zanonioideae), with 19 genera and 60 or more species (depending on species concepts in some Chinese genera, there could be as many as 90 species; Table 1), and the Cucurbitoidae, with 111 genera and c. 740 species. Nhandioboideae are characterized by a gynoeceum with three or rarely two, free styles, while Cucurbitoidae have the styles united into a single column (for additional characters of each subfamily see Section 4). The most recent classification recognizes two subfamilies and 11 tribes (Jeffrey, 2005; our Table 1). This classification, like the previous one (Jeffrey, 1990b), reflects the author's comprehensive knowledge of Cucurbitaceae worldwide and is "a product almost entirely of the intuitive, syncretistic approach" (Jeffrey, 1990a, p. 4). All higher taxa are based on combinations of traits.

Here, we rely on a combination of chloroplast genes, spacers, and introns to infer major clades in the family and to resolve their relationships among one another. Because this is the first molecular phylogeny for the family, we decided to focus on taxon sampling. We included 171 species of Cucurbitaceae that represent 123 of the 130 genera and all tribes and subtribes of recent classifications (Jeffrey, 2005; Jeffrey and De Wilde, 2006; De Wilde and Duyfjes, 2006a,b,e; our Table 1). A second goal was to provide the framework for a separate biogeographic study of Cucurbitaceae, which required adding species from specific areas. Cucurbitaceae contain many striking range disjunctions, such as those found in *Cayaponia*, *Kedrostis*, *Luffa*, *Sicyos*, and *Trichosanthes*, each with one or few isolated species in the New World, Africa/Madagascar, India, and/or Australia (assuming monophyly of these genera, an assumption here tested). Additional noteworthy range disjunctions may exist at the subtribal level. For example, in the smaller of the traditional subfamilies, Nhandioboideae, five subtribes are currently recognized (Table 1), one neotropical and containing seven species (Fevilleinae), two endemic in Asia and similarly small (Actinostemmatinae, Gomphogyninae), one pantropical and containing 28 species (Zanoniinae), and one disjunct between the Neotropics, East Africa, and Madagascar and containing 12 species (Sicydiinae; Jeffrey, 1990b, 2005). These and other groupings imply an astonishing number of over-water long distance dispersal events, unless they are assumed to all date back to at least 100 million years ago (mya).

Proper rooting of the Cucurbitaceae family tree requires that one know their closest relatives. The placement of Cucurbitaceae among Cucurbitales was therefore the focus of an earlier paper (Zhang et al., 2006), which sampled representatives of all seven families of the order (Anisophylleaceae, Begoniaceae, Coriariaceae, Corynocarpaceae, Cucurbitaceae, Datisceae, and Tetramelaceae) for nine loci from the chloroplast, nuclear, and mitochondrial genomes (together ~12,000 nucleotides). Cucurbitaceae were

well supported as sister to a clade of Begoniaceae, Datisceae, and Tetramelaceae, with the precise relationships not well-resolved. This does not present a problem for the current study because Datisceae and Tetramelaceae each have only two species, and Begoniaceae have only two genera, so that it is relatively easy appropriately to represent these families in a data matrix and to survey them for morphological character states of interest. The great genetic distance between Cucurbitaceae and their sister clade however, could lead to problems of long-branch attraction between outgroups and divergent ingroup members. We attempted to alleviate this problem by relying on maximum likelihood (ML) inference as well as parsimony, since ML is sometimes better able to accommodate rate heterogeneity (Sanderson and Shaffer, 2002 and references therein). Because of the large size of the data matrix, 174 taxa and 4917 included characters, we resorted to a new fast algorithm for maximum likelihood-based inference of phylogenetic trees (Stamatakis et al., 2005).

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

Table 2 lists all species sequenced for this study, their sources, GenBank accession numbers, and status as generic types where applicable. Tribal and subtribal assignments in the classification of Jeffrey (2005) are given in Table 1, which also includes five genera described recently (De Wilde and Duyfjes, 2006a,d; Jeffrey and De Wilde, 2006). Trees were rooted with a species each of Begoniaceae and Datisceae; a species of Corynocarpaceae was used as a more distant outgroup, based on Zhang et al. (2006).

Total genomic DNA was isolated from silica-dried leaves or from herbarium specimens with commercial plant DNA extraction kits (DNeasy, Qiagen; NucleoSpin, Machery-Nagel), following the manufacturers' manuals. The polymerase chain reaction (PCR) protocols used were as follows: Initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C for denaturation, 1 min for primer annealing at 48 °C for *rbcL*, 49 °C for *matK*, 53 °C for *rpl20-rps12* or 55 °C for the *trnL* region, and 1 min 40 s at 72 °C for DNA elongation, followed by a final elongation period of 7 min at 72 °C. Reactions were performed with 10 μM of primers, 25 μM MgCl₂, 1.25 μM of each dNTP, 2.5 μl of 10× PCR-buffer, 0.5 U *Taq* DNA polymerase, and 10–50 ng of template DNA per 25 μl reaction volume. Part of the PCR amplifications followed the protocol described in Zhang and Renner (2003). For recalcitrant material, we used more reactive polymerases (Phusion™ High Fidelity PCR Kit by Finnzymes; KOD Hot Start DNA Polymerase by Novagen) according to the manufacturers' protocols. Reaction products were purified with QIAquick gel extraction kits (Qiagen) or the Wizard SV gel and PCR clean-up kit (Promega), and cycle sequencing was performed with BigDye Terminator v3.1, v3.0 or v1.0 cycle sequencing kits (Applied Biosystems) using 1/4- or

Table 1

The most recent classification of Cucurbitaceae (Jeffrey, 2005)

Taxon (species number)	Distribution/comments	Fig. 1
I. Subfamily Nhandioboideae Kostel., 1833 (Zanonioidae C. Jeffrey, 1962a)		
Tribe Zanonieae Bl., 1826		
Subtribe Zanoniinae Pax, 1889		
<i>Alsomitra</i> (1)	West Malesia to E New Guinea	Yes
<i>Bayabusua</i> (3)	Malay Peninsula	Yes
<i>Gerrardanthus</i> (4)	Tropical and S Africa	Yes
<i>Nealsomitra</i> (12)	India, China, Polynesia, Australia	Yes
<i>Siolmatra</i> (2)	Amazon basin	Yes
<i>Xerosicyos</i> (2–3)	Madagascar	Yes
<i>Zanonia</i> (1)	India, China, Indochina, Malaysia, Philippines	—
<i>Zygosicyos</i> (2)	Madagascar	Yes
Subtribe Fevilleinae Pax, 1889		
<i>Fevillea</i> (7)	Central to tropical South America	Yes
Subtribe Gomphogyninae Pax, 1889		
<i>Gomphogyne</i> (1–2?)	China, Indochina, Malesian Region	—
<i>Gynostemma</i> (5–13)	India to Japan, Malaysia	Yes
<i>Hemsleya</i> (3–24)	China (Himalayan Mts.)	Yes
Subtribe Actinostemmatinae C. Jeffrey (1990b)		
<i>Actinostemma</i> (1)	NE China and Himalayan Mts., Japan	Yes
<i>Bolbostenma</i> (2)	China	Yes
Subtribe Sicydiinae Pax, 1889		
<i>Chalema</i> (1)	Mexico	Yes
<i>Cyclantheropsis</i> (2–3)	East Africa, Madagascar	Yes
<i>Pseudosicydium</i> (1)	Peru, Bolivia	—
<i>Pteropepon</i> (2?)	Brazil, Argentina	Yes
<i>Sicydium</i> (6)	Mexico, Central to tropical South America	Yes
II. Subfamily Cucurbitoidae Kostel., 1833		
1. Tribe Joliffieae Schrad., 1838 [Telfairieae Arn., 1841]		
Subtribe Telfairiinae Pax, 1889		
<i>Odosicyos</i> (1)	Madagascar	Yes
<i>Telfairia</i> (3)	Tropical Africa	Yes
Subtribe Thladianthinae Pax, 1889		
<i>Baijiana</i> (1)	Borneo	Yes
<i>Indofevillea</i> (1)	India, Bhutan, Tibet, Nepal	Yes
<i>Microlagenaria</i> (1)	Nigeria, Tanzania	Yes
<i>Momordica</i> (47)	Africa, trop. Asia, Australia; weedy in the Americas	Yes
<i>Sinobaijiana</i> (4) C. Jeffrey and De Wilde (2006)	China, Indonesia, Taiwan, Thailand	Yes
<i>Siraitia</i> (4)	India, China, Thailand, Vietnam, Indonesia	Yes
<i>Thladiantha</i> (25)	Tropical Asia	Yes
2. Tribe Bryoniae Dumort., 1827		
<i>Bryonia</i> (10)	Mediterranean to N Africa and central Asia	Yes
<i>Ecballium</i> (1)	Mediterranean to N Africa and central Asia	Yes
3. Tribe Trichosantheae (Pax) C. Jeffrey (1962a)		
Subtribe Ampelosicyinae C. Jeffrey (1962a)		
<i>Ampelosicyos</i> (3) [<i>Ampelosycios</i> Thouars, orth. var.]	Madagascar	Yes
<i>Tricyclandra</i> (1)	Madagascar	Yes
Subtribe Hodgsoniinae C. Jeffrey (1962a)		
<i>Hodgsonia</i> (2)	NE India to Borneo	Yes
Subtribe Trichosanthinae Pax, 1889		
<i>Gymnopetalum</i> (4) De Wilde and Duyfjes (2006c)	China, Indochina, India	Yes
<i>Trichosanthes</i> (100)	China, India, Malesia to Australia; one species, <i>T. amara</i> L., in Hispaniola	Yes
4. Tribe Herpetospermeae (C. Jeffrey) C. Jeffrey (2005)		
<i>Biswarea</i> (1)	India, Myanmar, China	Yes
<i>Edgaria</i> (1)	India, Nepal, China	Yes
<i>Herpetospermum</i> (1)	Nepal, Tibet, China	Yes
5. Tribe Schizopeponeae C. Jeffrey (1964)		
<i>Schizopepon</i> (8)	Russia, India, Myanmar, China, one species Japan	Yes
6. Tribe Luffeae (C. Jeffrey) C. Jeffrey (2005)		
<i>Luffa</i> (7)	Africa, Arabia, India, Asia, Australia (4 spp.), Central and South America (3 spp.)	Yes

(continued on next page)

Table 1 (continued)

Taxon (species number)	Distribution/comments	Fig. 1
7. Tribe Sicyeae Schrad., 1838		
Subtribe Cyclantherinae C. Jeffrey (1990b)		
<i>Brandegea</i> (1)	North America (Baja California)	Yes
<i>Cyclanthera</i> (including <i>Cremastopus</i>) (25)	North America (1) Mexico to Argentina	Yes
<i>Echinocystis</i> (1)	North America eastern N. America	Yes
<i>Echinopepon</i> (including <i>Apatzingania</i>) (18)	Arizona to N Argentina	Yes
<i>Elateriopsis</i> (5)	Central to tropical South America	Yes
<i>Hanburia</i> (2)	Mexico & Guatemala	Yes
<i>Marah</i> (7)	North America California to Oregon, adj. Mexico?	Yes
<i>Pseudocyclanthera</i> (1)	Paraguay, Bolivia	Yes
<i>Rytidostylis</i> (5)	Central to tropical South America	Yes
<i>Vaseyanthus</i> (1)	Mexico	Yes
Subtribe Sicyinae C. Jeffrey (1990b)		
<i>Microsechium</i> (2)	Mexico to Guatemala	Yes
<i>Parasicyos</i> (1)	Guatemala	Yes
<i>Sechiopsis</i> (including <i>Pterosicyos</i> ; Kearns (1992)) (5)	Mexico to Guatemala	Yes
<i>Sechium</i> (11)	Central to tropical South America	Yes
<i>Sicyos</i> (40)	North America to Argentina, Hawaii, 1 species in Australia and SW Pacific	Yes
<i>Sicyosperma</i> (1)	North America, extreme S Arizona	Yes
8. Tribe Coniandreae Endl., 1846		
<i>Apodanthera</i> (15)	North America (Texas), Sonoran Desert, to S. Am.	Yes
<i>Ceratosanthes</i> (4)	Central to tropical South America	Yes
<i>Corallocarpus</i> (17)	Africa, Madagascar, India	Yes
<i>Cucurbitella</i> (1)	South America (Bolivia)	Yes
<i>Dendrosicyos</i> (1)	Socotra Archipelago	Yes
<i>Dieterlea</i> (2, but see <i>Ibervillea</i>)	N. America	Yes
<i>Doyerea</i> (1)	Central to N South America	Yes
<i>Gurania</i> (35)	Central to tropical South America	Yes
<i>Guraniopsis</i> (1)	Peru	Yes
<i>Halosicyos</i> (1)	Argentina	Yes
<i>Helmontia</i> (1 or 2)	Guyana and Brazil	Yes
<i>Ibervillea</i> (including <i>Dieterlea</i> fide Kearns, 1994a; but not Jeffrey, 2005) (5)	North America (Texas), Mexico to Guatemala	Yes
<i>Kedrostis</i> (20–25)	Tropical and subtropical Africa, Madagascar (20), India, Sri Lanka, W Malesia (5)	Yes
<i>Melothrianthus</i> (1)	Brazil	Yes
<i>Psiguria</i> (12)	Central to tropical South America	Yes
<i>Seyrigia</i> (4)	Madagascar	Yes
<i>Trochomeriopsis</i> (1)	Madagascar	Yes
<i>Tumamoca</i> (1; Kearns (1994b))	North America (Arizona) to Mexico	Yes
<i>Wilbrandia</i> (5)	South America	Yes
9. Tribe Benincaseae Ser. 1825		
Subtribe Benincasinae (Ser.) C. Jeffrey (1962a)		
<i>Acanthosicyos</i> (2)	Angola, Namibia, Botswana, South African Republic	Yes
<i>Bambekea</i> (1)	Tropical Africa	Yes
<i>Benincasa</i> (1)	Asia, Australia	Yes
<i>Borneosicyos</i> (1)	Indonesia, Sabah	Yes
<i>Cephalopentandra</i> (1)	Tropical and subtropical Africa	Yes
<i>Citrullus</i> (3)	Tropical and subtropical Africa	Yes
<i>Coccinia</i> (30)	Tropical and subtropical Africa, Asia (1 sp.)	Yes
<i>Cogniauxia</i> (2)	Tropical Africa	Yes
<i>Ctenolepis</i> (2)	Tropical and subtropical Africa, India	Yes
<i>Dactyliandra</i> (2)	Tropical and subtropical Africa, India	Yes
<i>Diplocyclos</i> (4)	Tropical and subtropical Africa, Asia (1 sp.)	Yes
<i>Eureiandra</i> (8)	Tropical and subtropical Africa, Socotra	Yes
<i>Indomelothria</i> (2)	SE Asia	—
<i>Khmeriosicyos</i> (1)	Cambodia	—
<i>Lagenaria</i> (6)	Tropical and subtropical Africa, Asia	Yes
<i>Lemurosicyos</i> (1)	Madagascar	Yes
<i>Neoachmandra</i> (30)	Africa, Australia, Pacific Islands	Yes
<i>Nothalsomitra</i> (1)	Tropical Australia	Yes
<i>Papuasicycos</i> (1)	Papua New Guinea	—

Table 1 (continued)

Taxon (species number)	Distribution/comments	Fig. 1
<i>Peponium</i> (20)	Tropical Africa, Madagascar	Yes
<i>Praecitrullus</i> (1)	India, Pakistan	Yes
<i>Raphidiocystis</i> (5)	Tropical Africa, Madagascar	Yes
<i>Ruthalicia</i> (2)	Tropical Africa	Yes
<i>Scopellaria</i> (2)	SE Asia	Yes
<i>Solena</i> (3)	China, Indochina, India	Yes
<i>Trochomeria</i> (8)	Tropical and subtropical Africa	Yes
<i>Urceodiscus</i> (7)	New Guinea	—
<i>Zombitsia</i> (1)	Madagascar	Yes
Subtribe Cucumerinae Pax, 1889		
<i>Cucumella</i> (11)	Tropical Africa, Madagascar, India	Yes
<i>Cucumeropsis</i> (1)	Tropical Africa	Yes
<i>Cucumis</i> (33)	Tropical/subtropical Africa, Madagascar, Asia	Yes
<i>Dicaelospermum</i> (1); sunk into <i>Mukia</i> in De Wilde and Duyfjes (2006b)	India	Yes
<i>Melancium</i> (1)	Brazil	Yes
<i>Melothria</i> (10)	Tropical Central and South America	Yes
<i>Muellerargia</i> (2)	Madagascar, Australia (Queensland), Malaysia	Yes
<i>Mukia</i> (6)	Africa, SE Asia to N Australia	Yes
<i>Myrmecosicyos</i> (1)	Kenya	Yes
<i>Oreosyce</i> (1)	Tropical Africa, Madagascar	Yes
<i>Posadaea</i> (1)	Central and South America	Yes
<i>Zehneria</i> (25, sensu stricto)	Tropical/subtropical Africa, Madagascar, Asia	Yes
10. Tribe Cucurbitae Dumort., 1827		
<i>Abobra</i> (1)	South America	Yes
<i>Anacaona</i> (1)	Dominican Republic	Yes
<i>Calycophysum</i> (5)	Colombia to Bolivia	Yes
<i>Cayaponia</i> (60)	North America (1 sp.), Mexico to Argentina, W Africa (1–2 spp.), Madagascar (1 sp.)	Yes
<i>Cionosicyos</i> (3)	Central America, Jamaica	Yes
<i>Cucurbita</i> (20)	North America, Mexico to Argentina	Yes
<i>Penelopeia</i> (1)	Dominican Republic	Yes
<i>Peponopsis</i> (1)	Mexico	Yes
<i>Polyclathra</i> (1)	Mexico to Panama	Yes
<i>Schizocarpum</i> (6)	Mexico	Yes
<i>Selysia</i> (4)	Nicaragua to Peru	Yes
<i>Sicana</i> (3)	Mexico to Panama	Yes
<i>Tecunumania</i> (1)	Central America	Yes

Information on species numbers and geographic range is from monographs, floras, and our own studies of herbarium material.

1/8-scale reaction mixtures. The dye terminators were removed by 2 μ l of 3 mol/l NaOAc (pH 4.6) and 50 μ l ethanol precipitation or by Sephadex G-50 Superfine gel filtration (Amersham Biosciences) on MultiScreen TM-HV membrane plates (Millipore) according to the manufacturer's protocol. Purified sequencing reactions were run on an ABI Prism 3100 Avant, an ABI 3130 Genetic Analyzer or an ABI Prism 377 automated sequencer.

Primers used to amplify the *rbcL* gene were the same as in Zhang et al. (2006). For cycle sequencing, they were supplemented by the newly designed internal primers 600F (ATTTATGCGTTGGAGAGACCG) and 800R (CAA TAACRGCATGCATYGCACGRT). Primers for the *trnL* intron and adjacent *trnL-F* spacer and for the plastid *rpl20-rps12* spacer also were the same as in Zhang et al. (2006). In some cases, however, we used the newly designed primers *rpl20* 384F (TATACACCGGAGCTCYTTC) and/or *rpl20* 717R (GTTTCTATTGGTGAAATCC). The plastid maturase K (*matK*) gene was amplified with primers *matK-AF* and *matK-8R* (Ooi et al., 1995) and F1 and R1

(Yokoyama et al., 2000). For cycle sequencing, Yokoyama et al.'s AF, 8R, F1, R1, F3, and R3 were used. Many DNAs from herbarium material were amplified with low annealing temperatures and/or with internal primers. Forward and reverse reads were obtained for most samples. Sequences were edited with Sequencher (4.1–4.6; Gene Codes) and aligned by eye, using MacClade 4.06 (Maddison and Maddison, 2003).

2.2. Phylogenetic analyses

Parsimony searches were conducted with version 4.0b10 of PAUP (Swofford, 2002) and ML analyses with RAxML (Stamatakis et al., 2005), which implements a fast search algorithm for maximum likelihood-based inference of large phylogenetic trees. Computations were performed on the computer cluster of the 'CyberInfrastructure for Phylogenetic REsearch' project (CIPRES, www.phylo.org) at the San Diego Supercomputing Center. Parsimony analyses used the parsimony ratchet PRAP command block for

Table 2
Species and chloroplast regions sequenced, their sources, status as nomenclatural types, and GenBank Accession numbers

Species	DNA source	Geographic origin	<i>rbcL</i> gene	<i>matK</i> gene	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer
Ingroup Cucurbitaceae							
<i>Abobra tenuifolia</i> (Gillies ex Hook.) Cogn.	M. W. Chase 915 (K), Kew 1967-25606, aliquot	Brazil, Argentina, Uruguay	AF008961	DQ536629	DQ536782	DQ536782	DQ536616
<i>Acanthosicyos horridus</i> Welw. ex Benth. and Hook. f., GT	E. van Jaarsveld s.n., cult. Kirstenbosch BG	Angola, Namibia, N Cape Region	DQ535778	DQ536630	DQ536757	DQ536757	DQ536617
<i>Actinostemma tenerum</i> Griff., GT	(1) Wuyi Expedition 2490 (1981) (Z/ZT) (2) G. Murata 19027 (M) (3) Chinese collector (IBSC 244863)	(1) China, Wuyi Mt. (2) Japan: Honshu (3) China, Zhejiang	(1) DQ535779	(1) DQ536631	(1) DQ536783	(1) DQ536783	(1) DQ53652
<i>Alsomitra macrocarpa</i> (Bl.) M.J. Roem., GT	W. De Wilde and B. Duyfjes 21978 (L)	Tropical SE Asia	(3) DQ469135 ^a DQ535780	(3) DQ469136 ^a DQ536632	(2) DQ641904 ^a DQ536784	(2) DQ641904 ^a DQ536784	(3) DQ491007 ^a DQ536618
<i>Ampelosicyos humblotii</i> (Cogn.) Perrier et Jumelle	Fianarantsoa s.n. (P)	Madagascar	DQ501254	DQ521608	—	DQ501261	—
<i>Ampelosicyos scandens</i> Du Petit-Thouars, GT	(1) M. Keraudren 25372 (K), Kew 23790, aliquot (2) T. Croat 32220 (MO)	Madagascar	(1) EF066337	(1) EF066331	(1) EF066328	(1) EF066328	(1) EF066334
<i>Anacaona sphaerica</i> A. H. Liogier, GT	T. Zaroni et al. 39300 (NY)	Dominican Republic	DQ535781	DQ536756	DQ536785	(2) DQ535874 DQ536785	(2) DQ536527 DQ536528
<i>Apatzingania arachioidea</i> Dieterle, GT	Machuca 6547 (MEXU)	Mexico	DQ535739	DQ536633	DQ536786	DQ536786	DQ536529
<i>Apodanthera mandonii</i> Cogn.	M. and K. Weigend 2000/165 (M)	Peru, Cuzco	DQ535782	DQ536634	DQ536787	DQ536787	DQ536530
<i>Baijiantia borneensis</i> (Merrill) A.M. Lu and J.Q. Li, GT	A. D. E. Elmer 20472 (M)	Borneo	DQ535740	DQ469137	DQ501262	DQ501263	DQ491008
<i>Bambekea racemosa</i> Cogn., GT	J. Louis 13283 (M)	Africa: Congo	DQ535783	—	DQ536788	DQ536788	DQ536531
<i>Bayabusua clarkei</i> (King) W. De Wilde, GT	W. De Wilde and B. Duyfjes 21961 (L)	Peninsular Malaysia: Perak	DQ535741	DQ536635	DQ536758	DQ536758	DQ648155
<i>Benincasa hispida</i> (Thunb.) Cogn.	S. Renner et al. 2760 (M), cult. Mainz BG	SE Asia: prob. China	DQ535784	DQ536636	DQ536789	DQ536789	DQ536619
<i>Biswarea tonglensis</i> (C. B. Clarke) Cogn., GT	A. Stainton 8364 (E)	China	DQ535742	DQ536637	—	—	—
<i>Bolbostemma paniculatum</i> (Maxim.) Franquet	L. Ende and Y. Decai (KUN 0809223)	China, Yunnan, Yong De county	DQ501255	DQ469139	DQ501264	DQ501264	DQ491009
<i>Borneosicyos simplex</i> W. De Wilde, GT	SAN (Postar et al.) 144251 (L)	Sabah	DQ535785	DQ536638	DQ535869	DQ535877	DQ536620
<i>Brandegia bigelovii</i> (S. Watson) Cogn., GT	J. Buegge 1182 (ASU)	Arizona	DQ535866	DQ536639	DQ536790	DQ536790	DQ648156
<i>Bryonia alba</i> L., GT	S. Volz 6 (M)	Germany: Saxony	DQ535744	DQ536640	DQ533867	DQ533867	DQ536532
<i>Bryonia dioica</i> L.	S. Renner 2187 (M), cult. Zurich BG	Europe	DQ535786	DQ536641	DQ536791	DQ536791	DQ648157
<i>Calycophytum pedunculatum</i> H. Karst. and Triana, GT	(1) P. Acevedo-Rodriguez 8918 (G) (2) K. Young and Sullivan 700 (MO)	(1) Peru (2) Peru	(1) DQ535743	—	(1) DQ536792	(1) DQ536792	(2) DQ536533
<i>Cayaponia africana</i> (Hook. f.) Exell	E. Figueredo 249 (LISC)	São Tomé	DQ535787	DQ536642	DQ536759	DQ536759	DQ536621
<i>Cayaponia americana</i> (Lam.) Cogn.	C. Taylor 11784 (MO)	Florida	DQ535737	DQ536643	DQ536793	DQ536793	DQ648158
<i>Cayaponia podantha</i> Cogn.	Seeds from Hudson Seed Co., California, cult. B. Toskey	Argentina	DQ535738	DQ536644	DQ536760	DQ536760	DQ648159
<i>Cephalopentandra ecirrhosa</i> (Cogn.) C. Jeffrey	M. W. Chase 929 (K), aliquot from plant cult. at Kew 1977-3860, leg. Brandham 2400	Kenya, Kechilu Pass	AF534744	DQ536645	DQ536794	DQ536794	DQ648160

<i>Ceratosanthes palmata</i> (L.) Urb.	M. W. Chase 919 (K), Kew 1969-3534	Venezuela,	DQ535788	DQ536646	DQ536795	DQ536795	DQ536534
<i>Chalema synanthera</i> J.V.A. Dieterle, GT	V. W. Steinmann 3026 (NY)	Maracay, Mexico, Michoacán	DQ535789	DQ536647	DQ536796	DQ536796	DQ536535
<i>Cionostycus macranthus</i> (Pittier) C. Jeffrey	(1) E. Cruz, 6 May 2002, unvouchered (2) S. Swensen (aliquot)	1) Costa Rica: La Selva station	(1) DQ535790	(2) DQ536648	(1) DQ536797	(1) DQ536797	(1) DQ536536
<i>Citrullus colocynthis</i> (L.) Schrad.	S. Renner et al. 2762 (M), cult. Mainz BG	Cultivated worldwide	DQ535791	DQ536649	DQ536798	DQ536798	DQ648161
<i>Citrullus lanatus</i> (Thunb.) Matsum. and Nakai	Unvouchered store-bought water melon	Cultivated worldwide	DQ535745	DQ536650	DQ536761	DQ536761	DQ648162
<i>Coccinia grandis</i> (L.) Voigt, GT	W. De Wilde and B. Duyfjes 22270 (L)	Thailand	DQ535792	DQ536651	DQ536762	DQ536762	DQ536537
<i>Coccinia rehmannii</i> Cogn.	S. Renner and A. Kocyan 2749 (M), cult. Munich BG acc. 91/2485	Africa	DQ535793	DQ536652	DQ536799	DQ536799	DQ648163
<i>Coccinia sessilifolia</i> (Sond.) Cogn.	S. Renner et al. 2763 (M), cult. Mainz BG	Africa	AY968520	AY968446	AY968568	AY968385	AY968531
<i>Cogniauxia podolaena</i> Baill., GT	G. Walters and R. Niangadouma 1248 (MO)	Africa, Gabon: Haut-Ogooue	DQ535794	DQ536653	DQ536800	DQ536800	DQ536538
<i>Corallocarpus bainesii</i> (Hook. f.) A. Meese	H. H. Schmidt et al. 2294 (MO)	Zambia: Southern	DQ535795	DQ536654	DQ536801	DQ536801	DQ536539
<i>Corallocarpus boehmii</i> (Cogn.) C. Jeffrey	S. Renner et al. 2764 (M), cult. Mainz BG	Africa, Madagascar, India	DQ535796	DQ536655	DQ536802	DQ536802	DQ536540
<i>Ctenolepis cerasiformis</i> (Stocks) Clarke, GT	Seeds leg. M. Wilkins 279, leaf extracted by S. Swensen (aliquot)	Zimbabwe	DQ535797	DQ536656	DQ536803	DQ536803	DQ648164
<i>Cucumella bryoniifolia</i> (Merxm.) C. Jeffrey	Seeds leg. M. Wilkins 214b, cult. M. Wilkins	South Africa	DQ535798	DQ536657	DQ536763	DQ536763	DQ648165
<i>Cucumeropsis mannii</i> Naud., GT	G. Zenker 4648 (M)	Cameroon	DQ535746	—	—	DQ535875	DQ536541
<i>Cucumis hirsutus</i> Sond.	N.B. Zimba et al. 874 (MO)	Zambia: Southern	DQ535799	DQ536658	DQ536804	DQ536804	DQ536542
<i>Cucumis melo</i> L.	Unvouchered store-bought melon	Cultivated worldwide	DQ535800	DQ536659	DQ536764	DQ536764	DQ648166
<i>Cucumis metuliferus</i> E. Mey. ex Naud.	S. Renner et al. 2765 (M), cult. Mainz BG	Africa	DQ535801	DQ536660	DQ536805	DQ536805	DQ648167
<i>Cucumis sagittatus</i> Peyr.	D. Decker-Walters 1124 (FTG)	Namibia	DQ535802	DQ536661	DQ536806	DQ536806	DQ648168
<i>Cucumis sativus</i> L., GT	S. Renner 2745 (M), cult. Munich BG	Cultivated worldwide	DQ535747	DQ536662	DQ536765	DQ536765	DQ648169
<i>Cucumis zeyheri</i> Sond.	D. Decker-Walters 1114 (FTG)	Natal	DQ535803	DQ536663	DQ536807	DQ536807	DQ648170
<i>Cucurbita digitata</i> A. Gray	J. Buegge and Buegge 1181 (ASU)	Arizona	—	DQ536664	DQ535868	DQ535876	DQ536543
<i>Cucurbita ficifolia</i> Bouché	S. Renner et al. 2766 (M), cult. Mainz BG	Probably of New World origin	DQ535804	DQ536665	DQ535867	—	DQ648171
<i>Cucurbita okeechobeensis</i> ssp. <i>martinezii</i> (L.H. Bailey) Walters and Decker-Walters	O. Sanjur 76 (seeds); aliquot	Mexico: Veracruz	—	—	DQ536766	DQ536766	DQ536622
<i>Cucurbita pepo</i> L., GT	(1) Cult. Zurich BG, 2001 (2) GenBank	Cultivated worldwide	(2) L21938	(1) DQ536666	(1) DQ536808	(1) DQ536808	(1) DQ536623
<i>Cucurbitella asperata</i> (Hook. and Arn.) Walp., GT	M. Nee et al. 48807 (MO)	Bolivia	DQ535748	—	DQ536809	DQ536809	DQ536544
<i>Cyclanthera brachystachya</i> (Ser.) Cogn.	S. Renner et al. 2767 (M), cult. Mainz BG	Central and South America	DQ535749	DQ536667	DQ536767	DQ536767	DQ648172
<i>Cyclantheropsis parviflora</i> Harms, GT	J. B. Gillett 19443 (M)	Kenya	EF634363	EF634361	EF634364	EF634364	EF634362
<i>Dactylandra welwitschii</i> Hook. f., GT	Giess 3664 (M)	SW Africa	DQ535750	DQ536669	DQ536810	DQ536810	DQ536545

(continued on next page)

Table 2 (continued)

Species	DNA source	Geographic origin	<i>rbcL</i> gene	<i>matK</i> gene	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer
<i>Dendrosicyos socotranus</i> I. B. Balf., GT	(1) M. Olson s.n. (MO) (2) J. Lavranos s.n. (M), cult. Munich BG	Socotra	(2) AY973018	(2) AY973022	(2) AY973005	(2) AY973005	(1) AY968540
<i>Dicaeosperrmun ritchiei</i> C.B. Clarke, GT	H. Santapaa 13354 (MO)	India: Khandala	DQ535806	—	DQ536811	DQ536811	DQ536546
<i>Dieterlea fusiformis</i> E.J.Lott	Sukkulentensammlung Zürich, 93 1055/D; ex cult. Mostul 391	Mexico	—	—	DQ536812	DQ536812	—
<i>Dieterlea maxima</i> (Lira and Kearns) McVaugh (<i>Ibervillea maxima</i> Lira and Kearns)	M. Olson 842 (MEXU)	Mexico: Jalisco	DQ535807	DQ536670	DQ536813	DQ536813	DQ648173
<i>Diplocyclos palmatus</i> (L.) C. Jeffrey	J. Maxwell 2 Sep. 2002	Thailand: Chiang Mai	AY862552	DQ536671	DQ536769	DQ536769	DQ536625
<i>Doyerea emetocathartica</i> Grosourdy	R. Lira 471 (MO), 22 October 1983	Mexico	DQ535808	DQ536672	DQ535870	DQ535878	DQ536547
<i>Ecballium elaterium</i> (L.) A. Rich. ssp. <i>elaterium</i> , GT	(1) M. W. Chase 922 (K), Kew 1970-624; (2) S. Renner et al. 2768 (M), cult. Mainz BG	Europe	(2) AY973023	(2) AY973019	(2) AY973006	(2) AY973006	(1) AY968541
<i>Echinocystis lobata</i> (Michx.) Torr. and A. Gray, GT	S. Renner et al. 2829 (M), cult. Mainz BG	North America	DQ535809	DQ536673	DQ536814	DQ536814	DQ648174
<i>Echinopepon paniculatus</i> (Cogn.) Dieterle, Type not designated	R. Torres C. 14047 (M)	Mexico	DQ535810	DQ536674	DQ536815	DQ536815	DQ536548
<i>Echinopepon racemosus</i> (Steud.) C. Jeffrey	C. Taylor et al. 11350 (MO)	Argentina	DQ535751	DQ536675	DQ536770	DQ536770	DQ648175
<i>Echinopepon wrightii</i> (A. Gray) S. Watson	S. Renner 2808 (M), cult. Munich BG from seeds leg. M. Wilkins 446	Arizona	DQ535811	DQ536676	DQ536816	DQ536816	DQ536549
<i>Edgaria darjeelingensis</i> C.B. Clarke, GT	J. D. A. Stainton 1626 (G)	Nepal: Arun Valley	—	DQ536677	DQ536817	DQ536817	DQ536550
<i>Elateriopsis oerstedii</i> (Cogn.) Pittier	A. Jiménez M. 3961-A (G)	Costa Rica	DQ535752	DQ536678	—	—	DQ536551
<i>Eureiandra formosa</i> Hook. f., GT	(1) J. Lebrun 2929 (M)	Africa: Congo (both collections)	(1) DQ535812	—	(1) DQ641905 ^a	(1) DQ641905 ^a	(1) DQ536552
<i>Fevillea pergamentacea</i> (Kuntze) Cogn.	(2) J. Louis 10852 (M) Nee 52385 (NY)	Bolivia	(2) DQ641908 ^a	—	(2) DQ536818	(2) DQ536818	—
<i>Gerrardanthus grandiflorus</i> Gilg ex Cogn.	S. Renner 2717 (MO)	Tanzania	DQ535813	DQ536679	DQ536819	DQ536819	DQ536553
<i>Gerrardanthus macrorhizus</i> Harv. ex Benth. and Hook. f., GT	S. Renner et al. 2770 (M), cult. Mainz BG	South Africa	DQ535805	DQ536668	DQ536768	DQ536768	DQ536624
<i>Gurania makoyana</i> (Lem.) Cogn.	S. Renner et al. 2771 (M), cult. Mainz BG	Neotropics	DQ535753	—	DQ536820	DQ536820	DQ648176
<i>Gurania spinulosa</i> (Poepp. and Endl.) Cogn. (prob. same as <i>G. lobata</i> (L.) Pruski), LT	E. Cotton et al. 1742 (AAU)	Ecuador	AY973024	DQ536680	DQ536821	DQ536821	DQ648177
<i>Gurania tubulosa</i> Cogn. (including <i>G. megistantha</i> J.D. Sm.)	Cult. Missouri BG acc. 1993-1657-4	Costa Rica	—	AY968450	AY968569	AY968386	AY968542
<i>Guramiopsis longipedicellata</i> Cogn.	P. Hutchison 1152 (F)	Peru: Junin	DQ535816	DQ536682	DQ536823	DQ536823	DQ536555
<i>Gymnopetalum integrifolium</i> (Roxb.) Kurz	W. De Wilde and B. Duyfjes 22269 (L)	Thailand	DQ535754	DQ536683	DQ536824	DQ536824	DQ536556
<i>Gynostemma pentaphyllum</i> (Thunb.) Makino	H. Takahashi 20712 (GIFU)	Japan	AY968523	AY968451	AY973007	AY973007	AY968543
<i>Halosicyos ragonesei</i> Mart. Crov., GT	F. B. Vervoort 3589 (G)	Argentina: Catamarca	DQ535755	DQ536684	DQ535871	DQ535879	DQ536557
<i>Hanburia mexicana</i> Seem., GT	F. Ventura A. 15150 (MO)	Mexico	DQ535756	DQ536685	DQ536825	DQ536825	—
<i>Hemlontia leptantha</i> (Schltdl.) Cogn.	(1) R. Liesner 6673 (MO) (2) R. Oldeman B 4301 (P)	(1) Venezuela (2) French Guiana	(1) DQ535757	(2) DQ491025	(2) DQ521607	(2) DQ661616	(2) DQ661615

<i>Hemsleya heterosperma</i> (Wall.) C. Jeffrey (<i>Gomphogyne heterosperma</i> Wall.)	(1) A. Kocyan et al. AK166 (BKF) (2) Pooma et al. 3041 (L)	(1) Thailand: Chiang Mai (2) Thailand: Phatthana Nikhom District, Lop Buri	(1) DQ535817 (2) DQ641907 ^a	(1) DQ536686 (2) DQ641907 ^a	(1) DQ536826 (2) DQ641907 ^a	(1) DQ536826 (2) DQ641907 ^a	(1) DQ536558 (2) DQ641907 ^a
<i>Herpetospermum pedunculatum</i> (Ser.) Baillon, GT	Collector unknown, 15 October 1979 (B acc. 197/2004-1)	Nepal	DQ535818	DQ536687	DQ536827	DQ536827	DQ536559
<i>Hodgsonia heteroclita</i> (Roxb.) Hook.f. and Thoms., GT	R. Zhang 1 (M)	China: Guangdong	DQ535819	DQ536688	DQ536828	DQ536828	DQ536560
<i>Ibervillea hypoleuca</i> (Standl.) C. Jeffrey	Sukkulentensammlung Zürich ex cult. Mostul 808, 22366	Mexico	DQ535820	DQ536689	DQ536829	DQ536829	DQ536561
<i>Ibervillea lindheimeri</i> (A. Gray) Greene, GT	D. Kearns s.n., leaf extracted by S. Swensen (aliquot)	Texas	DQ535821	DQ536690	DQ536830	DQ536830	DQ648178
<i>Ibervillea millspaughii</i> (Cogn.) C. Jeffrey	D. Kearns 565, leaf extracted by S. Swensen (aliquot)	Mexico and Belize	DQ535822	DQ536691	DQ536831	DQ536831	DQ648179
<i>Ibervillea tenuisecta</i> (A. Gray) Small	Sukkulentensammlung Zürich, 97 1460/O; ex cult. Mostul 60	Mexico	—	—	DQ536832	DQ536832	—
<i>Indofevillea khasiana</i> Chatterjee, GT	T. Yandell s.n. (K), aliquot	India: Assam	DQ501256	DQ491016	DQ501265	DQ535883	DQ491010
<i>Kedrostis africana</i> (L.) Cogn., GT	Cult. Missouri BG acc. 1980-0781-1	Africa	DQ535823	DQ536692	DQ536833	DQ536833	DQ536626
<i>Kedrostis nana</i> (Lam.) Cogn.	M. W. Chase 274 (K), aliquot	Africa: From Nat'l Bot. Inst., Kirstenbosch	DQ535824	DQ536693	DQ536834	DQ536834	DQ536562
<i>Lagenaria breviflora</i> (Benth.) Roberty	M. Merello et al. 1331 (MO)	Africa: Ghana	AY935747	AY935934	AY968570	AY935788	AY973020
<i>Lagenaria siceraria</i> (Molina) Standl.	Cult. Missouri BG, 2002	Source unknown	DQ535825	DQ536694	DQ536771	DQ536771	DQ536627
<i>Lemurosicoy variegatus</i> (Cogn.) Keraudren, GT	D. J. Du Puy et al. M891 (P)	Madagascar	DQ501257	DQ491017	DQ501266	DQ501266	DQ491011
<i>Luffa acutangula</i> (L.) Roxb.	Cult. in Guangzhou BG, leg. L. X. Zhou s.n., 15. Apr. 2004	Asia	DQ535826	DQ536695	DQ536835	DQ536835	DQ536563
<i>Luffa cylindrica</i> (L.) Roem. (<i>L. aegyptiaca</i> P. Miller), GT	Cult. in Guangzhou GB, leg. L. X. Zhou s.n., 15. Apr. 2004	Asia	DQ535827	DQ536696	DQ536836	DQ536836	DQ536564
<i>Luffa operculata</i> L. (<i>L. quinquefida</i> (Hook. and Arn.) Seem.)	S. Renner and A. Kocyan 2754 (M), cult. from seeds from The Cuc. Network # 1440	Mexico	L21941	DQ536697	—	DQ535880	DQ536565
<i>Marah fabaceus</i> (Naud.) Greene	R. E. Ricklefs and S. Renner 1 (MO)	California	DQ535758	DQ536698	DQ536837	DQ536837	AY973021
<i>Marah macrocarpus</i> Greene	(1) D. Arisa and Swensen 1009 (RSA) (2) M. Olson s.n., 26. Nov. 2001 (MO)	Sonoran Desert	(1) AY968524	(1) AY968453	(2) AY968571	(2) AY968387	(2) DQ536566
<i>Melancium campestre</i> Naud., GT	G. Pabst et al. 8741 (M)	Brazil	—	—	DQ536838	DQ536838	DQ536567
<i>Melothria pendula</i> L., GT	E. Cotton et al. 1741 (AAU)	Ecuador	DQ535828	DQ536699	DQ536839	DQ536839	DQ536568
<i>Melothrianthus smilacifolius</i> (Cogn.) Mart. Crov., GT	H. S. Irwin et al. 28197 (MO)	Brazil	DQ535764	DQ536700	—	DQ535881	DQ536569
<i>Micrologenia africana</i> (C. Jeffrey) A.M. Lu and J.Q. Li, GT	H. Schlieben 5667 (M)	Tanzania: Lindi District	—	DQ491018	—	DQ501268	DQ491012
<i>Microsechium helleri</i> Cogn.	H. Förther 10430 (MSB)	Guatemala: Alta Verapaz	—	DQ536701	DQ536840	DQ536840	DQ536570
<i>Momordica calantha</i> Gilg	S. Renner 2715 (LE, MO)	Tanzania	DQ535759	DQ536702	DQ648193	DQ648193	DQ648180
<i>Momordica charantia</i> L.	S. Renner 2759 (M), plant grown from seeds bought in Colombo	Sri Lanka	DQ535760	DQ491019	DQ501269	DQ501269	DQ491013

(continued on next page)

Table 2 (continued)

Species	DNA source	Geographic origin	<i>rbcL</i> gene	<i>matK</i> gene	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer
<i>Momordica foetida</i> Schumach.	H. Schmidt et al., 1978 (MO)	Ghana	DQ535829	DQ536703	DQ536841	DQ536841	DQ648181
<i>Muellerargia timorensis</i> Cogn., GT	D. L. Jones 3666 (NE)	Australia: Queensland	DQ535777	DQ536704	DQ536842	DQ536842	DQ536571
<i>Mukia maderaspatana</i> (L.) M. Roem.	J. Maxwell 02-434 (CMU)	Thailand: Chiang Mai	DQ535761	DQ536705	DQ536843	DQ536843	DQ648182
<i>Myrmecosicyos messorius</i> C. Jeffr., GT	P. R. O. Bally B15187 (EA)	Kenya: Lake Elementaita	—	DQ536706	DQ535872	—	DQ536572
<i>Neochamandra indica</i> (Lour.) W. De Wilde and Duyfjes (<i>Zehneria indica</i> (Lour.) Keraudren)	X. F. Deng 171 (IBSC)	China: Guangdong	DQ535863	DQ536752	DQ536883	DQ536883	DQ536613
<i>Neochamandra japonica</i> (Thunb.) W. De Wilde and Duyfjes (<i>Zehneria japonica</i> (Thunb.) H. Y. Liu)	H. Takahashi 20764 (GIFU)	Japan	DQ535864	DQ536753	DQ536884	DQ536884	DQ648192
<i>Neosalsmitra capricornica</i> (F. Muell.) Hutch.	B.S. Wannan 305 (private herbarium)	Australia: Queensland	EF066338	EF066332	EF066329	EF066329	EF066335
<i>Neosalsmitra clavigera</i> (Wall.) Hutchinson	Phonsena et al. 4691 (L)	Thailand	DQ535830	DQ536707	DQ641901	DQ641901	DQ536573
<i>Neosalsmitra podagrica</i> Steenis	(1) S. Renner et al. 2777 (M), cult. Mainz BG (2) de Wilde and B. Duyfjes 21846 (L)	SE Asia	(2) DQ535831	(1) DQ536708	(2) DQ641902	(2) DQ641902	(2) DQ536574 (1) DQ674359
<i>Neosalsmitra sarcophylla</i> (Wall.) Hutchinson, GT	S. Renner et al. 2778 (M), cult. Mainz BG	SE Asia	AY968525	AY968454	AY973008	AY973008	AY968545
<i>Nothosalsmitra suberosa</i> (F.M. Bailey) I. Telford, GT	I. R. Telford 12487 (NE)	Australia: SE Queensland	DQ535762	DQ536709	DQ536844	DQ536844	DQ536575
<i>Odosicyos bosseri</i> Keraudren, GT	J. Bogner 2445 (M), cult. Munich BG acc. 90/784	Madagascar	DQ535832	DQ536710	DQ536773	DQ536773	DQ648183
<i>Oreosyce africana</i> Hook. f., GT	E. Phillips 2821 (Z)	Malawi	DQ535833	DQ536711	DQ536845	DQ536845	DQ536576
<i>Parasicyos dieterleae</i> Lira and Torres	A. García M. et al. 1704 (MO)	Mexico	DQ535763	DQ536712	DQ536846	DQ536846	DQ536577
<i>Penelopeia suburceolata</i> (Cogn.) Urban	A. Veloz et al. 1298 (B)	Dominican Rep., Prov. de la Vega	DQ535834	DQ536713	DQ536847	DQ536847	DQ536578
<i>Peponium caledonicum</i> (Sond.) Engl.	Seeds leg. M. Wilkins 405, cult. M. Wilkins	Namibia/S. Africa border	DQ535765	DQ536714	DQ536774	DQ536774	DQ536579
<i>Peponium vogelii</i> (Hook. f.) Engl.	S. Renner 2722 (LE)	Tanzania	DQ535835	DQ536715	DQ536775	DQ536775	DQ648184
<i>Peponopsis adhaerens</i> Naud., GT	T. C. Andres and J. J. Wyland 23 (MO)	Mexico	DQ535766	DQ536716	DQ536848	DQ536848	DQ536580
<i>Polyclathra cucumerina</i> Bertol., GT	M. Olson 812 (MEXU)	Mexico	DQ535767	DQ536717	DQ536849	DQ536849	DQ536628
<i>Posadaea sphaerocarpa</i> Cogn., GT	(1) M. Monsalve B. 579 (MO) (2) De Candolle herb. 1891, collector unknown (G)	Colombia: Valle del Cauca	(1) DQ535836 (2) DQ641909 ^a	(1) DQ536718	(1) DQ536850 (2) DQ641906 ^a	(1) DQ536850 (2) DQ641906 ^a	(1) DQ536581
<i>Praecitrullus fistulosus</i> (Stocks) Pangalo, GT	D. Decker-Walters 883 (FTG)	India	DQ535837	DQ536719	DQ536851	DQ536851	DQ648185
<i>Pseudocyclanthera australis</i> (Cogn.) Mart. Crov., GT	Elias de Paula 1834 (K), Kew 23793, aliquot	Paraguay	—	EF066333	—	EF066330	EF066336
<i>Psiguria racemosa</i> C. Jeffrey	Cult. Missouri BG acc. 1997-2683-2	Venezuela	DQ535735	DQ536720	DQ536852	DQ536852	DQ648186
<i>Psiguria umbrosa</i> (Kunth) C. Jeffrey	Cult. Missouri BG acc. 1997-0054-1	Venezuela	DQ535736	DQ536721	DQ536853	DQ536853	DQ648187
<i>Pteropepon parodi</i> Mart. Crov.	M. Nee et al. 52082 (NY)	Bolivia: Santa Cruz	DQ535838	DQ536722	DQ536854	DQ536854	DQ536582
<i>Raphidiocystis phyllocalyx</i> C. Jeffrey and Keraudren	Gilbert 2162 (M)	Africa: Zaire, Stanleyville	DQ535838	—	DQ536855	DQ536855	DQ536583

<i>Ruthalicia longipes</i> (Hook.f.) C. Jeffrey	J.J. Wieringa 5150	Gabon	DQ535840	DQ536723	DQ536856	DQ536856	DQ536584
<i>Rytidostylis ciliata</i> Kuntze	S. Renner and A. Kocyan 2752 (M), cult. Munich BG acc. 02/2539	South America	DQ535841	DQ536724	DQ536776	DQ536776	DQ648188
<i>Schizocarpum filiforme</i> Schrad., GT	(1) C. G. Pringle (M) (2) G. B. Hinton 9532 (G)	Mexico	(1) DQ535768	—	(1) DQ536857	(1) DQ536857	(2) DQ536585
<i>Schizocarpum palmeri</i> Cogn. and Rose	Kruse 2889 (M)	Mexico	DQ535769	DQ536725	DQ536858	DQ536858	DQ536586
<i>Schizocarpum reflexum</i> Rose	C. G. Pringle 13692 (M)	Mexico	DQ535770	—	DQ536859	DQ536859	DQ536587
<i>Schizopepon bryoniifolius</i> Maxim., GT	Cult. S. Renner from seeds leg. T. Fukuhara	Japan	AY973025	AY968456	AY973009	AY973009	AY968547
<i>Scopellaria marginata</i> (Bl.) W. De Wilde and Duyfjes (<i>Zehneria marginata</i> (Bl.) Keraudren)	A. Kocyan AK187 (BKF)	Thailand	DQ535865	DQ536754	DQ536885	DQ536885	DQ536614
<i>Sechiopsis tetraptera</i> Dieterle	J. Calónico S. 4793 (M)	Mexico: Jalisco	DQ535842	DQ536726	DQ536860	DQ536860	DQ536588
<i>Sechium edule</i> (Jacq.) Sw., GT	M. Olson 832 (MEXU)	Mexico	DQ535843	DQ536727	DQ536861	DQ536861	DQ536589
<i>Selysia prunifera</i> (Poepp. and Endl.) Cogn., GT	H. Hentrich FGIC60 (ULM)	French Guiana	DQ535844	DQ536728	DQ536862	DQ536862	DQ536590
<i>Seyrigia humbertii</i> Keraudren	Cult. Missouri BG acc. 1996-3485	Madagascar	AY968526	AY968457	AY973010	AY973010	AY968548
<i>Sicana odorifera</i> (Vell.) Naud., GT	S. Renner 2807 (M), cult. Munich BG from seeds leg. Decker-Walters 118016 (FTG)	Colombia	DQ535845	DQ536729	DQ536863	DQ536863	DQ536591
<i>Sicydium diffusum</i> Cogn.	R. Vasquez 13742 (MO)	Peru	DQ535771	DQ536730	DQ536864	DQ536864	DQ536592
<i>Sicydium tannifolium</i> (Kunth) Cogn.	T. Andres, Nee and Wyland 102 (MO) = AK148	Mexico	DQ535846	DQ536731	DQ536865	DQ536865	DQ536593
<i>Sicyos angulatus</i> L., GT	M. W. Chase 979 (K), Kew 1938-09807, aliquot	North America	DQ535847	DQ536732	DQ536777	DQ536777	DQ648189
<i>Sicyos baderoa</i> Hook. and Arn.	C. Heubl 01-045 (M), coll. 7.12.2004	South America: Chile	DQ535848	DQ536733	DQ536866	DQ536866	DQ536594
<i>Sicyosperma gracile</i> A. Gray, GT	M. Fishbein et al. 2565 (MO)	North America	DQ535772	DQ536734	DQ536867	DQ536867	DQ536595
<i>Sinobaijantia yunnanensis</i> (A. M. Lu and Z. Y. Zhang) C. Jeffrey and W. De Wilde (<i>Baijantia yunnanensis</i> (A.M. Lu and Z.Y. Zhang) A.M. Lu and J.Q. Li)	H. Schäfer 05/117 (M)	China: Yunnan	DQ501258	DQ469138	DQ501270	DQ501270	DQ491014
<i>Siolmatra brasiliensis</i> (Cogn.) Baill., GT	(1) Charpin, A. and L. Novara 23 016 (G) (2) Chase 22969 RBG Kew 1998-3503	(1) Argentina, Prov. Salta (2) Northeast Brazil	(1) DQ641910 ^a (2) DQ535849	(2) DQ536735	(2) DQ536868	(1) DQ641903 ^a (2) DQ536868	(1) DQ641911 ^a (2) DQ536596
<i>Siraitia grosvenorii</i> (Swingle) C. Jeffrey ex Lu and Z. Y. Zhang	Cao Ming s.n., Guangxi Botanical Garden, Aug. 2004	China: Guangxi	DQ535850	DQ536736	DQ536869	DQ536869	DQ536597
<i>Solena heterophylla</i> Lour., GT	A. Kocyan et al. AK191 (BKF)	Thailand: Chiang Mai	DQ535851	DQ536737	DQ536870	DQ536870	DQ536598
<i>Tecummania quetzalteca</i> Standl. and Steyermark, GT	L. D. Gómez 20988 (MO)	Costa Rica	DQ535852	DQ536738	DQ536871	DQ536871	DQ536599
<i>Telfairia occidentalis</i> Hook. f.	D. Decker-Walters 1133 (FTG)	Cameroon	DQ535773	DQ491020	DQ536872	DQ536872	DQ648190
<i>Telfairia pedata</i> (Sm. ex Sims) Hook. f., GT	J.A. Mlangwa et al. 1165 (MO)	Tanzania, Kilimanjaro	DQ535853	DQ491021	DQ501271	DQ501271	DQ374439
<i>Thladiantha davidii</i> Franch.	Hai He s.n., July 2001	China: Sichuan	DQ535854	DQ536739	DQ536778	DQ536778	DQ536600
<i>Thladiantha dubia</i> Bunge, GT	(1) S. Renner et al. 2780 (M), cult. Mainz BG (2) M. W. Chase 918 (K), Kew 1969-18665 aliquot	China: Precise origin unknown	(1) DQ535733 (2) DQ536740	(2) DQ536779	(2) DQ536779	(1) DQ536779	(1) DQ648191
<i>Thladiantha hookeri</i> C. B. Clarke	Maxwell s.n., 8 August 2002	Thailand: Chiang Mai	DQ535734	DQ491022	DQ536780	DQ536780	DQ536601

(continued on next page)

Table 2 (continued)

Species	DNA source	Geographic origin	<i>rbcL</i> gene	<i>matK</i> gene	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer
<i>Trichosanthes amara</i> L.	M. Mejia, J. Pimentel, and R. García 1877 (NY)	Dominican Republic	DQ535774	DQ536741	DQ536873	DQ536873	DQ536602
<i>Trichosanthes kirilowii</i> Maxim.	H. Takahashi 20711 (GIFU)	Japan	DQ535855	DQ536742	DQ536874	DQ536874	DQ536603
<i>Trichosanthes ovigera</i> Bl. (including <i>T. cucumeroides</i> (Ser.) Maxim. ex Franch. and Sav.)	H. Takahashi 20755 (GIFU)	Japan	DQ535856	DQ536743	DQ536875	DQ536875	DQ536604
<i>Trichosanthes reticulineris</i> C.Y. Wu ex S.K. Chen	X. F. Deng 131 (IBSC) = S. Renner silica	China: Guangdong	DQ535857	DQ536744	DQ536876	DQ536876	DQ536605
<i>Tricyclandra leandrii</i> Keraudren	Jongkind 3532 (WAG)	Madagascar	DQ501259	DQ491023	DQ501272	DQ501272	DQ491015
<i>Trochomeria macrocarpa</i> (Sond.) Hook. f.	W. Giess 13286 (M)	SW Africa	DQ535858	DQ536745	DQ536877	DQ536877	DQ536606
<i>Trochomeriopsis diversifolia</i> Cogn., GT	Sukkulentensammlung Zürich, 82 3861/0	Madagascar	DQ535859	DQ536746	DQ536878	DQ536878	DQ536607
<i>Tumamoca macdougallii</i> J. N. Rose, GT	F. W. Reichenbacher 1646 (MO)	USA: Arizona	DQ535860	DQ536747	DQ536879	DQ536879	DQ536608
<i>Vaseyanthus insularis</i> (S. Watson) Rose	E. J. Lott and T. H. Atkinson 2428 (MO)	Mexico	DQ535776	DQ536748	DQ536880	DQ536880	DQ536609
<i>Wilbrandia verticillata</i> Cogn.	P. Luetzelburg 12002 (M)	Brazil: Rio de Janeiro	DQ535861	DQ536749	DQ536881	DQ536881	DQ536610
<i>Xerosicyos danguyi</i> Humbert, GT	Cult. Missouri BG acc. 1984-0142	Madagascar	AY973026	AY968459	DQ536781	DQ536781	AY968550
<i>Xerosicyos pubescens</i> Keraudren	S. Renner and A. Kocyan 2750 (M), cult. Munich BG acc. 93/2428	Madagascar	DQ535775	DQ536750	DQ535873	DQ535882	DQ536611
<i>Zehmeria bodinieri</i> (H. Lév.) W. De Wilde and Duyfjes	A. Kocyan AK178 (BKF)	Thailand	DQ535862	DQ536751	DQ536882	DQ536882	DQ536612
<i>Zombitsia lucorum</i> Keraudren, GT	P. B. Phillipson 2541 (P)	Madagascar	DQ501260	DQ491024	DQ501273	DQ501273	—
<i>Zygocycos tripartitus</i> Humbert, GT	S. Renner and A. Kocyan 2751 (M), cult. Munich BG acc. 95/2716	Madagascar	DQ535732	DQ536755	DQ536886	DQ536886	DQ536615
Outgroups							
Begoniaceae							
<i>Begonia oxyloba</i> Welw. ex Hook. f.	(1) Hughes s.n. (L. Forrest 279) (E) (2) S. S. Renner 2716 (MO) (3) GenBank	Africa	(3) U59815	(1) AY968445	(1) AY968563	(2) AY968378	(1) AY968530
Datisceae							
<i>Datisca glomerata</i> (Presl) Baill.	(1) H. van der Werff 14002 (MO) (2) GenBank	(1) California, San Diego	(2) L21940	(1) AY968449	(1) AY968567	(1) AY968384	(1) AY968539
Corynocarpaceae							
<i>Corynocarpus laevigatus</i> J.R. Forst. and G. Forst.	(1) CHR herbarium acc. 420527; aliquot s. Wagstaff (2) GenBank	New Zealand: Totara, near Thames	(2) AF148994	(1) AY968448	(1) AY968565	(1) AY968382	(1) AY968537

The letters GT after a species name indicate that the species is the type of the respective genus; LT stands for lectotype; BG for botanical garden.

^a Sequenced, but not used in the final analysis.

PAUP (Müller, 2004), with 10 random taxon-addition replicates and tree-bisection-reconnection (TBR) swapping, with the ‘steepest descent’ option not in effect. Gaps were treated as missing data. The evolutionary model for ML analyses was selected from the 24 models implemented in MrModeltest 2.2 (Nylander, 2004), employing the Akaike information criterion. For the final combined data set (174 accessions), the best fit was the general time-reversible (GTR) model plus a gamma shape parameter (G) and proportion of invariable sites (P-Invar). RAxML does not implement P-Invar, and we therefore used the GTR + CAT approximation of the GTR + G model, which uses 25 rate categories instead of the four categories used in most other implementations of the gamma shape parameter for capturing rate heterogeneity (Stamatakis, 2006). Model parameters were estimated in RAxML over the duration of specified runs, and ML searches started either from complete random trees (40 searches) or from most parsimonious trees (40 searches) for a total of 80 ML searches.

Statistical support was measured by non-parametric bootstrapping as implemented in PAUP. Bootstrap proportions (BP) were based on 10,000 replicates, using a simple-taxon-addition tree as the starting point, TBR swapping, steepest descent not in effect, and one tree held in memory. More computationally intensive heuristic approaches have been found not to increase the reliability of bootstrapping (Müller, 2005). We consider nodes well supported that have a parsimony BP $\geq 70\%$ (Hillis and Bull, 1993). The final tree was drawn with TreeGraph (Müller and Müller, 2004).

3. Results

3.1. Locus lengths, base frequencies, inversions, and rate heterogeneity

Chloroplast sequences were generated for 171 species of Cucurbitaceae from 123 genera, i.e., 21% of the family’s species and 95% of its genera. (Representatives of *Cyclantheropsis* and *Pseudocyclanthera* were added after this paper had been accepted.) The final concatenated matrix contained 5376 characters of which 4917 were included in the analyses. Twenty-six of the final 174 species lacked sequences for one or more of the loci (Table 2). Final alignments (available from TreeBASE, <http://www.treebase.org/treebase/>, submission number SN2921) comprised 1662 aligned positions from the *trnL* region, 1001 from the *rpl20-rps12* spacer, 1208 from the *matK* gene (positions 106 [1st codon position] to 1323 [3rd codon position] as compared to the tobacco *matK* GenBank accession AB240139), and 1350 included positions from the *rbcL* gene (positions 71 [1st codon position] to 1420 [3rd codon position] compared to the tobacco *rbcL* GenBank Accession No.: M16867). Excluded from all analyses were a poly(T) run (nine nucleotides [nt]) of *matK*, a 150-nucleotide section of repeated AT motifs in the *trnL* intron, one poly-A/G run (together 58 nt) in the *trnL* intron, two 5-nt-long regions and one 29-nt-long region in the *trnL-F*

spacer, plus a poly(A) run of 29 nt and a poly(T) run of 27 nt in the *rpl20-rps12* spacer.

To assess the homogeneity of base frequencies across taxa, we ran χ^2 tests in PAUP for each of the individual data sets, excluding missing or ambiguous sites. Results for the four data partitions were: $\chi^2 = 86.96$, $df = 504$, $P = 1.0$ for the *trnL* region; $\chi^2 = 86.01$, $df = 486$, $P = 1.0$ for *rpl20-rps12*; $\chi^2 = 103.26$, $df = 456$, $P = 1.0$ for *matK*; $\chi^2 = 43.43$, $df = 477$, $P = 1.00$ for *rbcL*. None of the tests revealed nucleotide bias among taxa. Empirical base frequencies in the 174 taxon file were A = 0.30, C = 0.18, G = 0.19, T = 0.33.

An inversion of 35–40 nt was found in the *trnL-F* spacer, about 30 nt upstream from the –35 bacterial-type promoter element for the tRNA^{Phe} gene (Steinmetz et al., 1983). The relevant region is involved in hairpin formation by intra-strand base pairing (Fig. 2). Its orientation in most Cucurbitaceae is shown in Fig. 2a. In four species of *Neoalsomitra* (Fig. 2b; *Neoalsomitra capricornica* was added after this paper went to press) and in one of the four species of *Cucurbita* (*Cucurbita digitata*; Fig. 2c), this hairpin was independently inverted again. At that time, *Cucurbita* had already acquired a simple sequence repeat of GAAAT (compare Fig. 2c and d, this repeat is found only in species of *Cucurbita*). We reverse-complemented all inverted 35–40 nt and aligned them with the outgroups so that their autapomorphic and synapomorphic mutations affected parsimony and ML analyses.

The *rpl20-rps12* spacer contained 217 (23%) informative sites (190 just for the ingroup), the *trnL* region 302 (21%; 274 just for the ingroup), the *matK* gene 378 (32%; 345 just for the ingroup), and the *rbcL* gene 198 (15%; 184 just for the ingroup). Topologies resulting from the individual datasets yielded no well-supported conflicting nodes, and we therefore combined the four data sets. The concatenated data yielded 1958 equally parsimonious trees (Consistency Index = 0.55, Retention Index = 0.77; statistics excluding autapomorphies).

The gamma shape parameter estimated by RAxML (in 80 ML searches that started from different starting trees; see Section 2) was between 0.42 and 0.44, indicating strong rate heterogeneity. About half the parsimony trees and ten of the 20 highest likelihood trees showed *Alsomitra macrocarpa*, a species traditionally assigned to Nhandioboideae, as sister to all other Cucurbitaceae. The other half of the parsimony and ML trees exhibited the topology illustrated in Fig. 1 (which shows the tree with the best likelihood score) in which Nhandioboideae are monophyletic. With outgroups excluded and the tree rooted with a member of the ingroup, *Indofevillea*, a monotypic genus sister to the rest of Cucurbitaceae (Fig. 1), Nhandioboideae receive 100% bootstrap support and Cucurbitaceae (except *Indofevillea*) 75%.

3.2. Phylogenetic relationships

Parsimony and maximum likelihood yielded topologies that were identical except for the following placements (all statistically poorly supported): (i) *Ruthalicia longipes*

under ML was sister to the remaining Old World Benincaseae (Fig. 1), while under parsimony it was sister to the clade including *Coccinia* and *Neoachmandra*; (ii) *Echinocystis lobata* (Sicyeae) under ML was sister to *Marah* (Fig. 1), while under parsimony it was sister to *Hanburia*; and (iii) Herpetospermeae/Schizopeponeae under parsimony were sister to the clade comprising *Nothoalsomitra* and Sicyeae, while under ML they were sister to the rest of the Fused Stamen clade (Fig. 1). There was no statistical support for the precise relationships between American and African Coniandreae (Fig. 1) or for the placement of Bryonieae and Herpetospermeae/Schizopeponeae relative to Benincaseae, Cucurbitaeae, and Coniandreae (labeled CBC clade in Fig. 1).

Our preferred topology (Fig. 1) fits with the traditional subdivision of Cucurbitaceae into Cucurbitoideae and Nhandiroboideae. Molecular data also recover eight of the 11 tribes circumscribed in Jeffrey's (2005) classification, but almost none of the subtribes (Table 1 vs. Fig. 1; also Section 4). Highly polyphyletic tribes are Joliffieae and Trichosantheae (sensu Jeffrey, 2005). The genera of Joliffieae form a grade at the base of Cucurbitoideae, and embedded among them are *Cogniauxia* of the traditional Benincaseae, and *Ampelosicyos* and *Tricyclandra* of the Trichosantheae. Most other Trichosantheae form a grade basal to Sicyeae. An unexpected finding is that *Indofevillea* is sister to all other members of Cucurbitoideae (also Section 4).

Of the genera sampled for more than one species, several are poly- or paraphyletic. Most important among these is *Cucumis* of which we included six species, chosen to represent its morphological range (Kirkbride, 1993; better species sampling in *Cucumis* confirmed the present findings, Renner et al., 2007): *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* were nested among species of *Cucumis*. Of *Ibervillea*, we included four species and found both species of the genus *Dieterlea* nested among them. Of *Psiguria*, we included two species and found *Gurania* and *Hemontia* nested among them (implications of this for character evolution are discussed under Section 4.4.5). Of the mostly Asian genus *Trichosanthes*, we included four species, including its sole New World species *Trichosanthes amara* from Hispaniola, and discovered that *T. amara* is closer to New World Sicyeae than to the remaining species of *Trichosanthes*, implying that *Trichosanthes* as currently circumscribed is paraphyletic. *Ampelosicyos*, sampled for two species, has *Odosicyos* and *Tricyclandra* embedded within in; *Neoachmandra*, also sampled for two species, has a species of *Zehneria* nested within it; and *Xerosicyos*, sampled for two of its possibly three species, has a species of *Zygosityos* falling within it.

4. Discussion

4.1. Possible impact of missing taxa and characters

The genera of Cucurbitaceae not represented in our sampling (Table 1) are all small or monotypic. They

are *Khmeriosicyos* De Wilde and Duyfjes, a monotypic genus from Cambodia only known from the type (De Wilde et al., 2004), *Indomelothria* De Wilde and Duyfjes, two species from Myanmar to west Malesia, *Papuasicyos* Duyfjes, a single species from Papua New Guinea (Duyfjes et al., 2003), and *Urceodiscus* De Wilde and Duyfjes, seven species from New Guinea. They all have fused styles or filaments and are therefore expected to fall in the Benincaseae (De Wilde et al., 2004; De Wilde and Duyfjes, 2006a). *Gomphogyne* W. Griffith, a genus with two or more species from Indochina and China, and *Zanonia* L., a monotypic genus ranging from India across Indochina to tropical China, Indonesia, and the Philippines, have three free styles and are therefore expected to belong in the Old World Nhandiroboideae. *Pseudosicydium* Harms, with a single species from Peru and Bolivia, also has free styles and thus is expected to place with the New World Nhandiroboideae (Jeffrey, 1990b, 2005).

Our combined data matrix contains 5% missing characters, and 26 species lack a sequence for one or more of the loci. Simulations suggest that the inaccurate placement of incomplete taxa is not usually the result of missing data but rather of an insufficient number of (parsimony) informative characters (Wiens, 2003, 2005; Philippe et al., 2004). Still, the presence of missing cells affects estimates of model parameters, especially if unevenly distributed across the data matrix. We therefore relied on parsimony inference as well as model-based maximum likelihood inference. Simulation and theory also indicate that bootstrap proportions tend to underestimate accuracy when a clade is correct (Zharkikh and Li, 1992a,b; Hillis and Bull, 1993; Felsenstein and Kishino, 1993; Efron et al., 1996). When discussing the implications of our findings (below), we concentrate on well-supported clades ($\geq 70\%$ BP) and discuss less well-supported groups only where they are of exceptional morphological or biogeographic interest.

4.2. An inversion in the *trnL-F* spacer as a molecular synapomorphy of Cucurbitaceae

Comparison with the Cucurbitales *trnL-F* alignment of Zhang et al. (2006; TreeBASE Accession Nos. s1392 and M2494–M2504) showed that the 35 or 40 nt-long inversion in the *trnL-F* intergenic spacer of Cucurbitaceae (Fig. 2) is not present in other Cucurbitales. Its occurrence is therefore a synapomorphy of Cucurbitaceae, with independent reversals in *Neoalsomitra* (Nhandiroboideae) and in *C. digitata* (Cucurbitaeae of Cucurbitoideae; Fig. 1). That these latter reversals happened independent of each other is evident not only from the phylogenetic positions of these species (as inferred from other substitutions), but also from the simple GAAAT sequence repeat present in both species of *Cucurbita* (Fig. 2c and d), which implies that the inversion in the *Cucurbita* lineage happened after *Cucurbita* had acquired the GAAAT repeat.

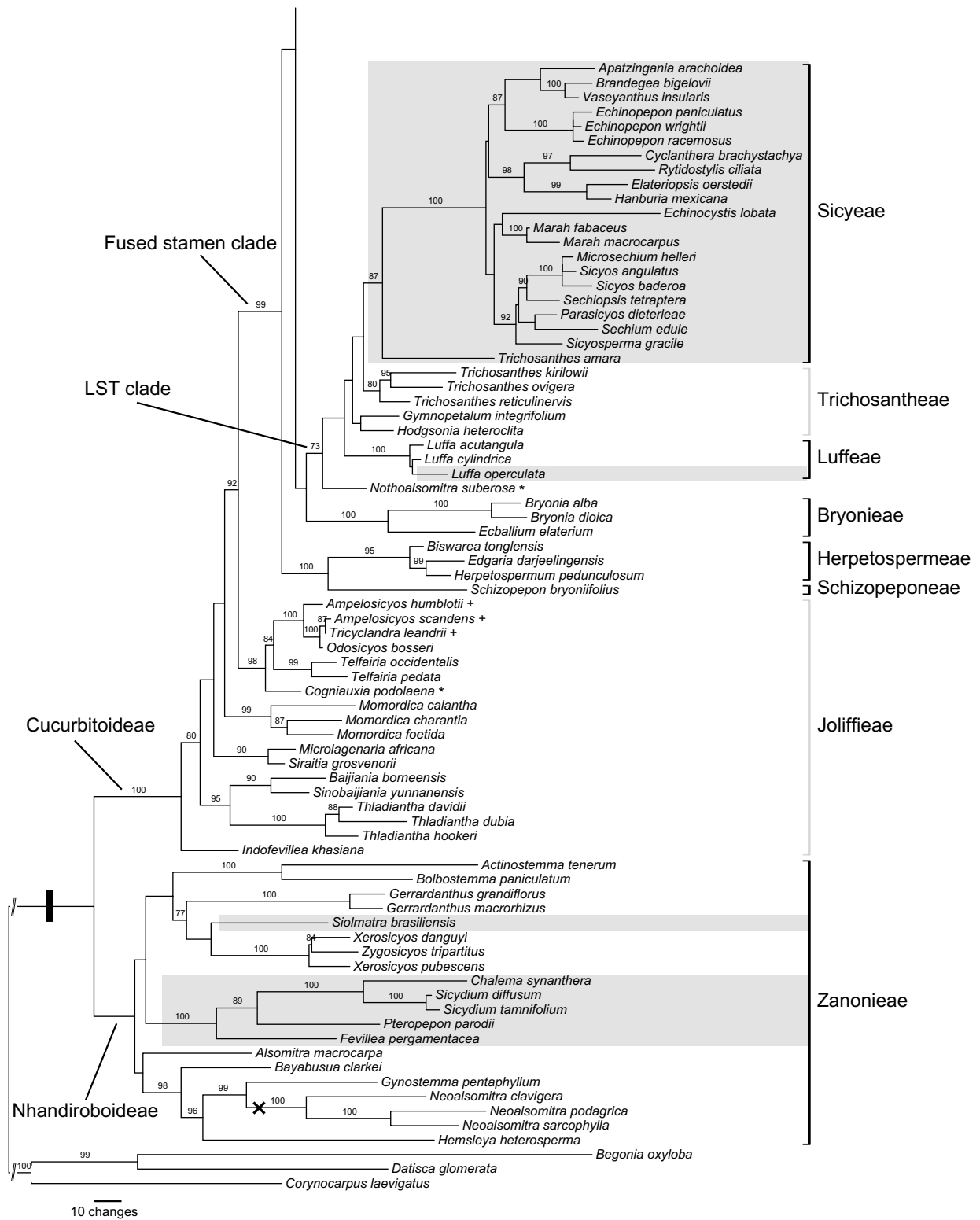


Fig. 1. One of the highest-scoring likelihood trees under the GTR + G model for Cucurbitaceae obtained from combined chloroplast loci (*matK*, *rbcL*, the *trnL* region, and *rpl20-rps12*), with parsimony bootstrap proportions shown above branches. Genera marked by an asterisk (*) were placed in Benincaseae by Jeffrey (2005), but are placed differently here (*Bambekea*, *Cogniauxia*, *Cucumeropsis*, *Eureiandra*, *Nothoalsomitra*); genera marked by a cross (+) (*Ampelosicyos* and *Tricyclandra*) were placed in Trichosantheae, but also are placed differently here. The bar at the base of Cucurbitaceae indicates the gain of a 35-nt-long inversion in the tRNA^{Leu}-tRNA^{Phe} intergenic spacer (compare Fig. 2); the X refers to two reversals of the inversion. Of the monotypic genera obtained after this paper went to press, *Cyclantheropsis parviflora* is sister to *Chalema* and *Sicydium* in the Zanonieae, and *Pseudocyclanthera australis* is sister to *Rytidostylis* in the Sicyeae. The tribal classification follows Jeffrey (2005), and the black brackets refer to clades, the grey brackets to grades.

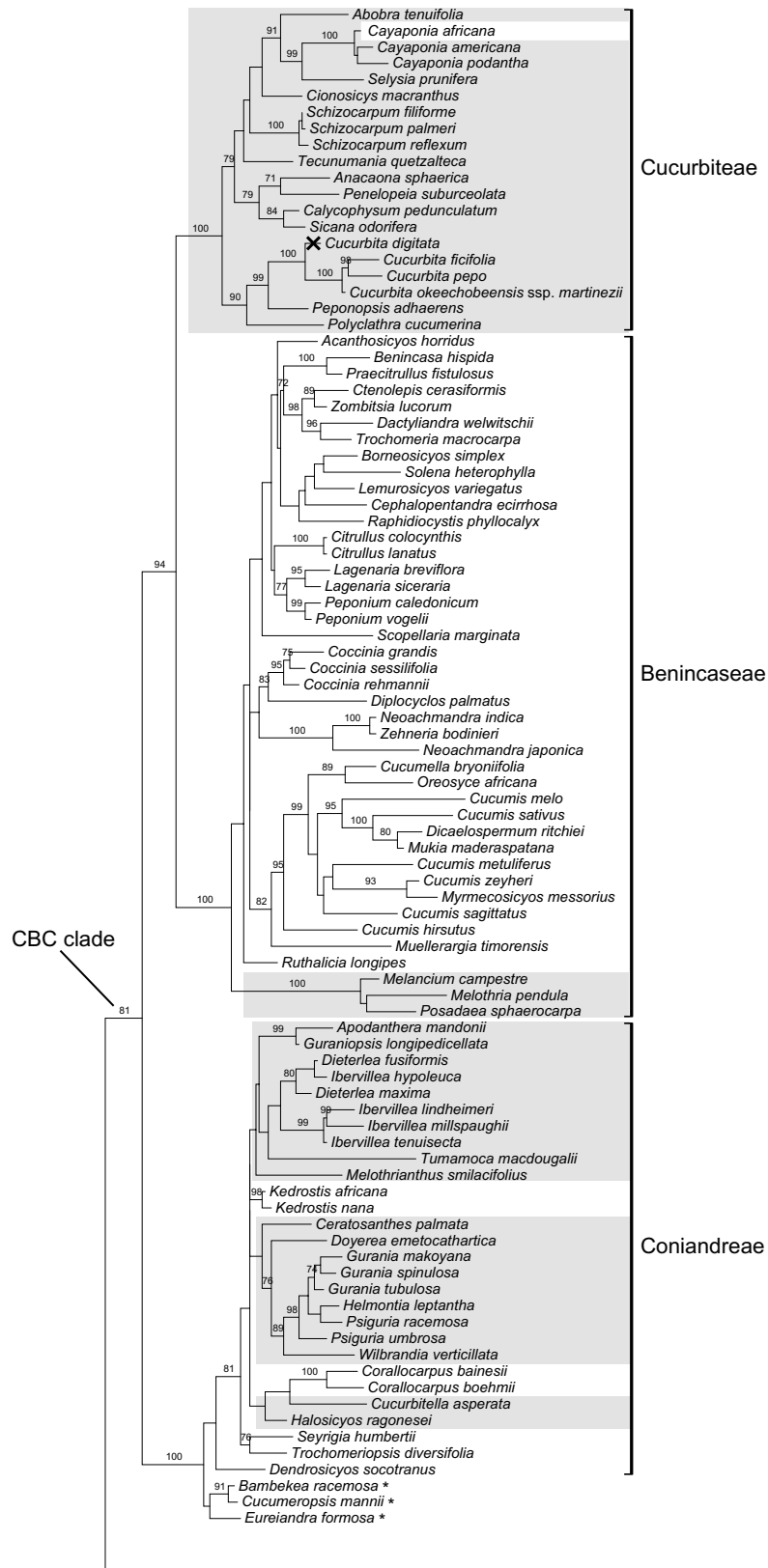


Fig. 1 (continued)

4.3. Major clades of Cucurbitaceae

Our results support the subdivision of Cucurbitaceae into two subfamilies, although only one of them, Cucurbitaceae, is statistically supported by the current gene, spacer, and intron sequences. Parsimony and ML searches both also recovered alternative topologies in which subfamily Nhandioboideae was paraphyletic and one of its genera, *Alsomitra*, was sister to all other Cucurbitaceae. An earlier analysis that involved 11 representatives of the main lineages of Cucurbitaceae sequenced for nine combined loci from the nucleus, chloroplast, and mitochondrion (Zhang et al., 2006) found weak support for the monophyly of Nhandioboideae (53–57% jackknife support, 88% ML jackknife support) and strong support for that of Cucurbitaceae (100% BP). *Alsomitra macrocarpa* is a woody climber that is widespread from west Malesia to eastern New Guinea. It can attain 50 m in length, with a stem base of up to 15 cm in diameter. Its critical morphological characters, such as tendril type, flower morphology, and pollen type, are those of a ‘normal’ nhandioboid cucurbit (Cogniaux, 1881; Troll, 1939; Jeffrey, 1962a; Duyfjes and De Wilde, 1998). Given (i) the morphological support for a Nhandioboideae clade (next section), (ii) the long branches leading to *Alsomitra* and to the three outgroups, and (iii) the rate heterogeneity in the data indicated by the small value of the gamma shape parameter (0.42–0.44), the placement of *A. macrocarpa* as sister to all other Cucurbitaceae may result from long-branch attraction.

4.3.1. Nhandioboideae

This subfamily comprises 60 species in 19 genera, of which we sampled all but three. Morphologically, this subfamily is characterized by free styles (usually two or three, rarely one), pendulous ovules, and small pollen grains with a striate exine. Most nhandioboids have branched tendrils with a sensitive basal part, the so-called zanonoid tendrils (see Section 4.4.1), and this may be another shared derived trait. The male flowers usually have five free stamens or three free filaments (due to fusion of two neighboring pairs of stamens) yet five free anthers (due to incomplete fusion of the thecae although the number of pollen sacs often is reduced; see Section 4.4.4). *Fevillea*, with seven species, is the only genus of Cucurbitaceae that retains the presumably ancestral condition of five free stamens with bilocular anthers (Robinson and Wunderlin, 2005b).

Within Nhandioboideae, subtribes Gomphogyninae and Zanoninae (as circumscribed by Jeffrey, 2005; our Table 1) are polyphyletic, while Sicydiinae, characterized by 1-seeded indehiscent fruits, appear to be monophyletic. The distinctive operculate fruits of *Actinostemma* and *Bolbostemma* (Jeffrey, 1990b, 2005) also evolved but once in their common ancestor (Fig. 1). The genus *Nealsomitra*, traditionally placed in Zanoninae (Jeffrey, 2005) and here embedded among two genera of Gomphogyninae (Fig. 1), is unusual in comprising species with free stamens as well as species with connate stamens (De Wilde and Duyfjes,

2003b), yet so far seems monophyletic. The most unexpected finding concerning Nhandioboideae, however, is that *Siolmatra*, a genus of two species in the Amazon basin (Robinson and Wunderlin, 2005a), is sister to a Madagascan clade comprising *Xerosicyos* (three species) and *Zygosicyos* (two species); the Indian/Southeast Asian *Zanonia*, which has not yet been sequenced, however, may modify this group. That *Siolmatra* has its closest relatives in the Old World was also recognized based on morphology (Cogniaux, 1881, p. 930), and indeed *Siolmatra brasiliensis* was originally placed in the above-discussed Malesian genus *Alsomitra*; Jeffrey, 1962a,c, 2005). These relationships probably imply a Gondwanan disjunction.

4.3.2. Cucurbitaceae

The 111 genera and c. 740 species of this subfamily all share a gynoeceum with a single style. The subfamily is currently subdivided into ten tribes (Jeffrey, 2005), six of which are supported by molecular characters (Benincaseae, Bryoniae, Coniandreae, Cucurbitaceae, Herpetospermeae, Sicyeae), although minor adjustments are required to make Benincaseae and Coniandreae fully monophyletic (Fig. 1). The monogeneric tribe Schizopeponeae (*Schizopepon*; eight species) based on our data might well be merged with Herpetospermeae (Fig. 1), while Joliffieae and Trichosantheae clearly are polyphyletic. Each of the main groups (tribes) is briefly discussed in the next sections, with a view to identifying where morphological homology assessments will need to be reconsidered.

The deepest divergence within Cucurbitaceae lies between *Indofevillea* and all other Cucurbitaceae. *Indofevillea* comprises a single species, *I. khasiana*, a dioecious climber in India, Bhutan, Tibet, and Nepal. The female flowers are unknown so that it is unclear whether *Indofevillea* has the single style that is typical of Cucurbitaceae or the three styles typical of Nhandioboideae. In its zanonoid tendril and androeceum with five stamens it resembles Nhandioboideae (see Sections 4.4.1 and 4.4.4). Its reticulate pollen exine, however, is more typical of Cucurbitaceae than of Nhandioboideae, which often have a striate exine (see Section 4.4.5).

Indofevillea is followed by a grade comprising the nine genera of Joliffieae sensu Jeffrey (2005), plus *Sinobaijiana*; Table 1 and Fig. 1. The Joliffieae were always “rather heterogeneous [...] and] in many ways the least specialized of the Cucurbitaceae” (Jeffrey, 1980) and “not very satisfactorily defined” (Jeffrey, 1990a). Their two subtribes, Telfairiinae (*Odosicyos*, *Telfairia*) and Thladianthinae (*Baijiana*, *Indofevillea*, *Microlagenaria*, *Momordica*, *Sinobaijiana*, *Siraitia*, *Thladiantha*), both are polyphyletic (at least as circumscribed in Jeffrey, 2005). These subtribes were based on fringed vs. unfringed petals, with or without basal scales. Similar fringed petals evolved several times (e.g., in *Hodgsonia* (De Wilde and Duyfjes, 2001), *Ampelosicyos*, *Tricyclandra*, *Trichosanthes*). Instead of grouping by petal characters, genera sort geographically: For example, *Odosicyos* of Jeffrey’s Telfairiinae, with a single species in

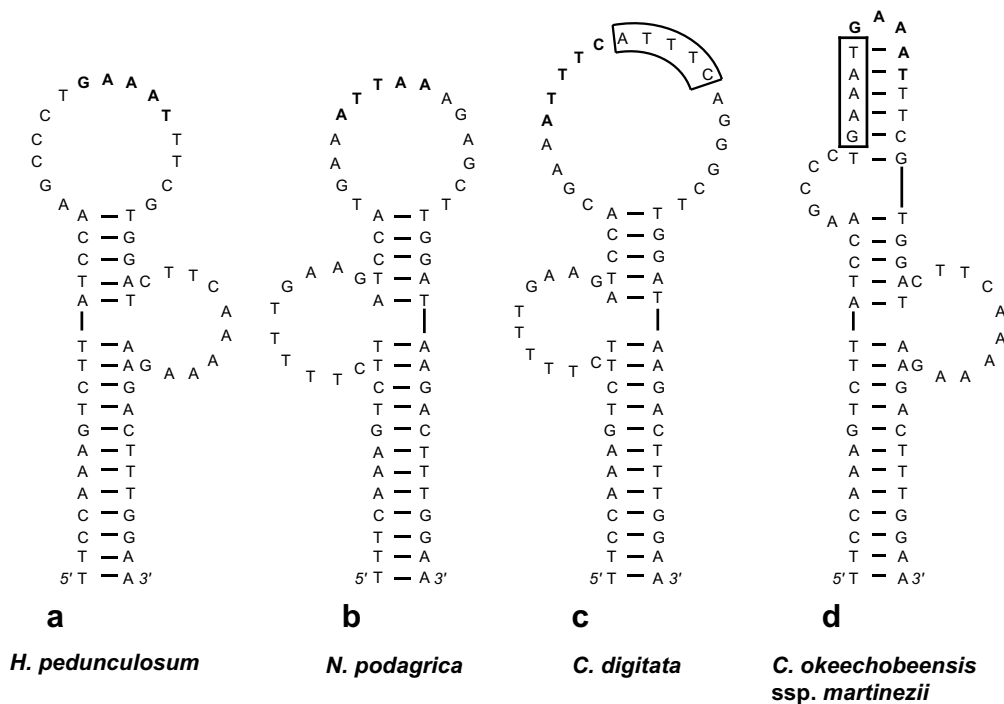


Fig. 2. Secondary structure models of an inversion of 35 or 40 nt found in the tRNA^{Leu}-tRNA^{Phe} intergenic spacer, just upstream from the –35 promoter. (a) *Herpetospermum pedunculosum*, an example of the stem and loop found in most Cucurbitaceae. (b) *Neoalsomitra podagrifica*, an example of the inverted loop found in all four species of *Neoalsomitra*. (c) *Cucurbita digitata*, with a re-inverted loop found only in this species. (d) *Cucurbita okeechobeensis*, with a stem and loop homologous to that of *Herpetospermum* and most other Cucurbitaceae. Boxes surround a 5-nt simple sequence repeat gained in the common ancestor of *Cucurbita* before the 40-nt stretch was inverted again in *C. digitata*. Nucleotides in bold represent the repeated sequence.

Madagascar, groups with other Madagascan genera, namely *Ampelosicyos* and *Tricyclandra* (Fig. 1). All three are sister to *Telfairia* (Ivory Coast to Tanzania), and the next-closest taxon is *Cogniauxia*, with two species, *C. podolaena* from western tropical Africa (Cameroon to Angola; sequenced) and *C. trilobata* from the Congo region (not yet sequenced). Similarly, *Thladiantha* does not group with the African *Microlagenaria*, but instead with the Asian *Baijiania* and *Sinobaijiania*.

The Joliffieae grade (Fig. 1) is followed by a clade that is recovered in all 20 highest likelihood trees and which we here refer to as the *Fused Stamen* clade (marked in Fig. 1). It is characterized by postgenitally or congenitally fused filaments and connectives (see Section 4.4.4). The deepest divergence within the *Fused Stamen* clade consists of a statistically unresolved polytomy comprising (i) Herpetospermeae/Schizopeponeae, (ii) Bryonieae, (iii) the CBC clade (Coniandreae (Benincaseae, Cucurbitae)), and (iv) *Nothoalsomitra*, *Luffa*, *Gymnopetalum*, *Hodgsonia*, *Trichosanthes*, and Sicyeae (Fig. 1). We refer to this last clade as the LST clade after the initials of its best-known components (*Luffa*, Sicyeae, *Trichosanthes*) and discuss it below because it implies a previously unsuspected Asian/American connection.

The relatively poorly known Schizopeponeae/Herpetospermeae (Fig. 1) comprise *Schizopepon* with eight species in Russia, Korea, Japan, and the Sino-Himalayan region (Lu, 1985) and *Herpetospermum*, *Biswarea*, and *Edgaria*,

each with single species in the Sino-Himalayan region. The seed coats of the last three genera contain a layer of cells with osteosclereids instead of the more common astrosclereids (Jeffrey, 2005), and their pollen is consistently 3-porate with a baculate exine, a combination of characters setting them apart from other Cucurbitaceae.

Bryonieae comprise only *Ecballium* with one species and *Bryonia* with 10 or 12 (Jeffrey, 1969; S. Volz, personal communication, 31 March 2006). *Bryonia* and *Ecballium* are endemic to the Mediterranean, Macaronesian, and Irano-Turanian floristic regions, with two species of *Bryonia* extending into northern Europe. Seeds assigned to *Bryonia* have been reported from the Middle Miocene of Western Siberia (Dorofeev, 1963, 1988), and Bryonieae likely represent a remnant of the tropical flora that occupied the northern Tethys border during the Eocene and Oligocene; their phylogeographic history is the focus of ongoing research (S. Volz, Ph.D. dissertation, University of Munich).

The LST clade has almost 300 species (Table 1), half of them in the Old World, the remaining in the New World, where Sicyeae have c. 125 species. At the base of the LST clade may be *Nothoalsomitra*, with a single species from eastern Australia, or *Luffa*, with four species in Africa, Arabia, India, Southeast Asia, and Australia, and three in Central and South America (Heiser and Schilling, 1990; Chung et al., 2003; we sampled *Luffa operculata* (*Luffa quinquefida*) from Mexico, *Luffa acutangula* of

Indian origin, and *Luffa cylindrica* of Australasian origin). When describing the genus *Nothoalsomitra* for an unusual species from mountain forests in SE Queensland, Telford (1982) placed it in the Benincaseae because of its globose synandrium with strongly sigmoid thecae, resembling those of more “derived” Cucurbitaceae. Within that tribe, *Nothoalsomitra* has been seen as close to *Borneosicyos* (De Wilde et al., 2004). Based on the chloroplast sequences, however, it places far from Benincaseae, and study of fertile material is now needed for a better assessment of key traits, such as ovule orientation and pollen exine and aperture type.

The largest genus in the LST clade is *Trichosanthes*, with 100 species in Asia and one in the New World (below), also by far the largest genus in the family. *Trichosanthes* has long been seen as closest to the Asian genera *Hodgsonia* (Müller and Pax, 1889) or *Gymnopetalum* (Jeffrey, 1962a; De Wilde and Duyfjes, 2006c), and also close to African/Madagascan groups such as *Peponium* (Jeffrey, 1962a; see also Table 1). Molecular data, however, show that the Trichosantheae Jeffrey are a polyphyletic mix of genera (Fig. 1). The Madagascan ‘Trichosantheae’, *Ampelosicyos* and *Tricyclandra*, group with other Madagascan genera (of former Joliffieae) as already discussed above. The remaining Asian ‘Trichosantheae’ form a grade (Fig. 1) below the Sicyeae, with the astonishing finding that *Trichosanthes amara* L. from Hispaniola (Dominican Republic and Haiti) is sister to all other Sicyeae. The genus *Trichosanthes* is otherwise restricted to eastern Asia, tropical Australia, and Fiji (Jeffrey, 1980, 1990b; Rugayah and De Wilde, 1997, 1999). The Hispaniolan species of the genus described by Linnaeus was never excluded from *Trichosanthes* (W. de Wilde, Leiden, personal communication, March 2006; C. Jeffrey, St. Petersburg, personal communication, March 2006, suspected that the species did not belong in *Trichosanthes*, but did not make the transfer). Based on molecular data, *T. amara* should be accorded generic rank, as also suggested by Liogier (1986, pp. 325–326) who studied the few existing collections.

The last component of the LST clade is the Sicyeae (125 species). Morphologically, this New World group is characterized by filaments united into a central column (Jeffrey, 1962a), nectaries derived from secretory trichomes on the hypanthium (Vogel, 1981; Jeffrey, 1990a), and 4- to 16-colporate or -colpate pollen (Stafford and Sutton, 1994; Section 4.4.5). The traditional subtribe Sicyinae, defined by single-seeded fruits, is embedded in subtribe Cyclantherinae (Jeffrey, 2005; our Table 1 and Fig. 1), which has many-seeded fruits. As found in another molecular study (H. Cross, personal communication, 2005), *Sicyos* appears to be paraphyletic (compare our Fig. 1, where the two species of *Sicyos* are in a polytomy with *Microsechium*).

Seed coat characters were used by Jeffrey (2005) to re-circumscribe Coniandreae to include 19 genera, and this is strongly supported by molecular data (Fig. 1). Coniandreae seeds lack a hypodermis, while other Cucurbitaceae have seed coats with a hypodermis of one or many layers of scler-

otic cells (Singh and Dathan, 1974, 2001, and references cited therein). Based on our results, *Bambekea*, *Cucumeropsis*, and *Eureiandra*, which Jeffrey (2005) still placed in Benincaseae, instead may be sister to the Coniandreae (but this only has 68% bootstrap support) and probably should be included in that tribe (Fig. 1). Sectioning of the seed coat showed that *Cucumeropsis mannii* has a well-developed hypodermis (Hanno Schaefer, personal observation; no seeds of the other two genera could be investigated), and the loss of a hypodermis therefore appears to have occurred in the stem lineage of Coniandreae proper (sensu Jeffrey, 2005). Remarkably, the “tree” genus *Dendrosicyos* (really an extreme pachycaul; Olson, 2003), with the single species *D. socotranus* on the Gondwanan fragment Socotra, is sister to all other Coniandreae. The relationships between the New World and African Coniandreae are not solidly resolved by our data (Fig. 1), which fits with the morphological similarities between these genera pointed out by previous workers (e.g., Jeffrey, 1962a, 1978). Thus, generic boundaries are unclear between *Kedrostis*, with 20 species (of which we sampled two), and *Corallocarpus*, with 17 species (of which we also sampled two), and between *Apodanthera* and *Guraniopsis* (which form a clade; Fig. 1), and *Cucurbitella*, *Melothrianthus*, and *Wilbrandia* (Jeffrey, 1962a; Pozner, 1998; De Wilde and Duyfjes, 2004).

The Benincaseae of Jeffrey (2005) become monophyletic after the exclusion of *Bambekea*, *Cucumeropsis*, *Eureiandra*, *Nothoalsomitra*, and *Cogniauxia* (Fig. 1). Their traditional subtribes, Benincasinae and Cucumerinae, are highly polyphyletic (compare Table 1 and Fig. 1). It is indicative of the long interaction between man and Cucurbitaceae that the clade’s name-giving taxon, *Benincasa*, with a single species, may only occur in cultivation, although Telford (1982) reports an apparently native population of *Benincasa hispida* from NE Queensland. The recent subdivision of *Zehneria* into five genera (*Indomelothria*, *Neoachmandra*, *Scopellaria*, *Urceodiscus*, and *Zehneria* s.str.; De Wilde and Duyfjes, 2006a,d), is partly supported (Fig. 1; *Indomelothria* and *Urceodiscus* have not yet been sequenced). There is additional evidence from pollen characters that some African species of *Melothria* or *Zehneria*, such as the Ethiopian–Madagascan species *Melothria peneyana* (Naud.) Cogn. (*Zehneria peneyana* (Naud.) Asher and Schweinfurth) with unusual 6-zonoporate pollen (Keraudren-Aymonin et al., 1984; Van der Ham and Pruesapan, 2006), may also be misplaced.

The last of the 10 tribes of Cucurbitoideae, Cucurbiteae, is monophyletic as traditionally circumscribed (Jeffrey, 1971, 1980, 2005). They are characterized by large spiny pantoporate pollen (see Section 4.4.5) and confined to the New World except for two species of *Cayaponia* in West Africa and Madagascar (*Cayaponia africana*, which we sequenced, and *Cayaponia multiglandulosa*, which may be the same species), which may stem from a long distance dispersal event. The monotypic genera *Penelopeia* and *Anacana* from the eastern part of Hispaniola (i.e., the Dominican Republic) are sister to one another, and this

divergence provides a maximum age constraint for a molecular-clock. (A biogeographic analysis with molecular clock-based age estimates is underway.)

4.4. Implications for morphological homologies and parallelisms

The following five sections discuss the implications of the molecular tree for the evolution of characters that have traditionally played a role in the higher-level classification of Cucurbitaceae. We also specify instances where a reinvestigation is required because character states previously considered homologous were apparently acquired independently.

4.4.1. Climbing, tendrils, and pachypodia

Tendrils, which in Cucurbitaceae are modified shoots (Lassnig, 1997), are the clearest macromorphological synapomorphy of the family Cucurbitaceae. They are present in all but a few derived, often woody taxa, where they have been transformed into thorns (*Acanthosicyos horridus*, *A. naudiniana*, *Momordica spinosa*) or completely lost (*Citrullus ecirrhosus*, *Dendrosicyos socotranus*, *Ecballium elaterium*, *Melancium campestre*, *Myrmecosicyos messorius*, *Trochomeria polymorpha*). Most authors distinguish (i) simple tendrils, (ii) branched tendrils with an insensitive basal part that does not coil, and (iii) branched tendrils with a coiling basal part (Troll, 1939; Jeffrey, 1962a, 1967; Lassnig, 1997). Branched tendrils with a touch-sensitive coiling base are traditionally referred to as zanonoid tendrils because they characterize most Zanonieae, the sole tribe of Nhandioboideae (younger synonym: Zanonioideae). In Cucurbitaceae, the ability to coil below the branching point is only found in a few basal members. Most Cucurbitaceae instead have simple, bifid, or multifid tendrils with up to eight branches (for example, the Benincaseae *Benincasa*, the Cucurbitaceae *Cayaponia* and *Sicana*, and the LST clade members *Echinocystis*, *Echinopepon*, *Hodgsonia*, and *Trichosanthes*). Adhesive patches, similar to those of *Parthenocissus* in the Vitaceae, have evolved in several species, including *Alsomitra macrocarpa* (Troll, 1939; Duyfjes and De Wilde, 1998), *Bayabusua clarkei* (De Wilde and Duyfjes, 1999; Hanno Schaefer, personal observation), *Nealsomitra sarcophylla*, *Polyclathra cucumerina* (McVaugh, 2001), and *Trichosanthes cucumerina* (G. Hausner, Wiesbaden, personal communication, December 2005).

Another striking feature of Cucurbitaceae is pachycauly. The evolution of pachypodia (conical above-ground tubers) in climbers is known from a few other angiosperm families, including Apocynaceae, Dioscoreaceae, Icacinaceae, Passifloraceae, and Vitaceae, but appears especially common in Cucurbitaceae. It is thought to be an adaptation to xeric environments where rocky substrates make the formation of belowground water-storing root systems difficult (Olson, 2003), and it is exactly in such habitats that perennial cucurbits often

have enlarged bases and pachypodia. No phylogenetic pattern is apparent; pachypodia are found in Nhandioboideae (*Gerrardanthus*, *Nealsomitra*, *Xerosicyos*, *Zygosityos*) as well as Cucurbitaceae, e.g., Benincaseae (*Cephalopentandra*, *Coccinia*, *Trochomeria*), Coniandreae (*Corallocarpus*, *Doyerea*, *Ibervillea*, *Kedrostis*, *Seyrigia*), Sicyeae (*Marah*), and *Sinobaijiana*, *Momordica*, *Odosicyos*, and *Tricyclandra*. *Dendrosicyos* is an extreme case of pachycauly (Olson, 2003).

4.4.2. Suppression of male or female organs, and gynoecium evolution

Flowers of Cucurbitaceae are usually diclinous (unisexual), and of the circa 800 species in this family, 460 are monoecious, 340 dioecious. Some species produce a mixture of bisexual, female, and male flowers in various intra- and inter-individual patterns, and populations can be andromonoecious, androdioecious, gynomonocious or gynodioecious (e.g., Whitaker, 1933; Jeffrey, 1969, 1980; Perl-Treves, 1999; Kater et al., 2001). During the development of a cucumber flower, organ primordia of all four whorls (sepals, petals, stamens, and carpels) are initiated (Kater et al., 2001), but at about 6 days, either the stamens or the carpels begin to expand rapidly. Analysis of cucumber floral homeotic mutants demonstrated that inhibition of stamens or pistils depends on whorl position, not specific sexual organ identity (Kater et al., 2001).

The gynoecia of Cucurbitaceae consist of one to five carpels, the tricarpellate condition being the most common. A phylogenetically highly conserved character is the number of styles: Nhandioboideae usually have three, sometimes two, free styles, whereas Cucurbitaceae have a single style with two, three or five stigmas, which are often enlarged and then mimic an androecium, probably to attract pollen-seeking bees who have already visited male flowers (Dukas, 1987; Rust et al., 2003). Ovule orientation in the Nhandioboideae is exclusively pendulous, and the number of ovules is relatively small (Section 4.4.3). By contrast, Cucurbitaceae have ascending or horizontal ovules (but rarely also pendulous), large placentas, and occasionally huge ovule numbers (Matthews and Endress, 2004; a tabulation of gynoecia characters by genus is available from the first author).

4.4.3. Fruit evolution

The fruits of Cucurbitaceae are poorly represented in collections because they are often difficult to collect and preserve. As a result, the mature fruits of quite a few species and genera, including *Helmontia*, *Sinobaijiana yunnanensis* (De Wilde and Duyfjes, 2003a; Jeffrey and De Wilde, 2006; Tables 1 and 2), and *Odosicyos* (Rauh, 1998) remain unknown or have been collected but a few times. The commercially important species of Cucurbitaceae (all in subfamily Cucurbitaceae) often have hard-shelled berries, called pepos, or gourds, that can reach huge dimensions (up to 1 m diameter have been recorded in *Cucurbita pepo*).

In the seasonally dry habitats, where most of the pepo-fruited species occur, hard-shelled, water-storing fruits allow for prolonged protected seed maturation, which can still take place even after the remainder of the vegetative shoot has mostly dried out and died off. Capsules releasing winged seeds adapted for wind dispersal predominate in Nhandioboideae (*Alsomitra*, *Fevillea*, *Gerrardanthus*, *Neoalsomitra*, *Pseudosicydium*, *Zanonia*). Many-seeded fruits have been inferred to be the ancestral condition in Begoniaceae, Cucurbitaceae, Datisceae, and Tetramelaceae (Zhang et al., 2006), while 1-seeded fruits evolved secondarily, for example, in *Hodgsonia* (De Wilde and Duyfjes, 2001), Sicydiinae, and the Sicyeae *Sechium* and *Sicyos*. Other Sicyeae have explosive fruits (*Cyclanthera*) or subterranean ones (in the Mexican genus *Apatzingania*); geocarpic fruits also evolved in the Benincaseae *Cucumis humifructus* (Kirkbride, 1993; Renner et al., 2007). Based on the molecular tree (Fig. 1), fruit characters appear evolutionarily highly plastic, and of the higher-ranked clades, such as tribes, none have a uniquely characteristic fruit type.

4.4.4. Androecium evolution

The androecium of Cucurbitaceae ancestrally consists of five stamens, the filaments of which can be free or united into a column. Where all five stamens are free and structurally identical, the androecium is completely actinomorphic, as in *Fevillea*, and this may represent the plesiomorphic condition. Increasing fusion between neighboring stamens results in androecia that have three stamens (two double stamens and a single stamen), four stamens (via the loss of one) or two stamens (below). The tendency for stamens to fuse is found in both subfamilies, but is more pronounced in Cucurbitoideae.

Nhandioboideae typically have five free stamens or three free filaments (due to fusion of two neighboring pairs of stamens) yet five anthers because of incomplete fusion of the thecae (*Alsomitra*, *Bayabusua*, *Pteropepon*, *Sicydium*). Three of their genera, *Gerrardanthus*, *Xerosicyos*, and *Zygosicyos*, have four stamens, but only the last two form a clade, while *Gerrardanthus* is only distantly related to them. This agrees with the androecia of *Xerosicyos* and *Zygosicyos*, which are truly 4-merous, while in *Gerrardanthus* a fifth stamen is present as a staminode (Cogniaux, 1916, p. 18). *Cyclantheropsis* (sequenced after this paper went to press) has an autapomorphic androecium in which the filaments form a column that bears two or three horizontally orientated anthers with a ring-like dehiscence line; it is sister to *Sicydium* and *Chalema*. A similar anther head evolved independently in subfamily Cucurbitoideae (Sicyeae: *Cyclanthera*).

Cucurbitoideae mostly have three stamens, but floral developmental studies have shown that even in flowers with three stamens, five distinct stamen primordia are initiated that alternate with the petals and of which four unite in a pair-wise manner while the fifth remains single (Matthews and Endress, 2004 and references therein). Early-branching Cucurbitoideae, such as *Indofevillea* and the genera of the

Joliffieae grade (Fig. 1), still have five stamens, four of which are pair-wisely loosely fused at their bases (*Baijiania*, *Siraitia*, *Thladiantha*). The basal members of the *Fused Stamen* clade and most genera in the LST clade (Fig. 1) have androecia in which the filaments of two pairs of stamens are completely fused while the fifth stamen remains free. A staminal column evolved in some Sicyeae (*Cyclanthera*, *Echinopepon*, *Rytidostylis*; Pozner, 2004). The remaining Cucurbitoideae, that is, Benincaseae, Coniandreae, and Cucurbiteae (the CBC clade in Fig. 1), have three or two stamens or rarely a staminal column (the *Anacaona/Penelopeia* clade; Liogier, 1986). Two stamens are especially common in Coniandreae, where they characterize *Gurania*, *Psiguria*, and *Helmontia* (Fig. 1). Two stamens evolved a second time in the *Apodanthera/Guraniopsis* clade (Fig. 1). Anthers in Benincaseae, Coniandreae, and Cucurbiteae can be free (e.g., *Cephalopentandra*, Benincaseae) or fused and extremely folded (e.g., *Cucurbita*, Cucurbiteae), and anthers from one and the same androecium can have one, two, three, or four pollen locules. Understanding evolutionary trajectories will require dense species sequencing and new anatomical-developmental work. However, a family-wide evolutionary trend appears to be staminal fusion and enlargement of the pollen-producing space through sigmoid coiling of the locules. This is more pronounced in Cucurbitoideae than in Nhandioboideae and may relate to the large pollen grains that characterize Cucurbitoideae (see Section 4.4.5).

4.4.5. Pollen evolution

Pollen morphology in Cucurbitaceae has received considerable attention (Marticorena, 1963; Jeffrey, 1964; Stafford and Sutton, 1994; Khunwasi, 1998; Van der Ham, 1999; Van der Ham and Van Heuven, 2003; Barth et al., 2005; Pruesapan and Van der Ham, 2005; H. Halbritter, A. Kocyan, M. Hesse, S. S. Renner, unpublished data), and molecular data support the perception that pollen characters in Cucurbitaceae are relatively conserved.

In general, pollen of Cucurbitaceae is tectate to semitectate, and grains are shed as monads, rarely as tetrads (below). Nhandioboideae pollen is always tricolporate and has a diameter of usually less than 40 μm . The exine is usually striate (17/19 [investigated genera/total genera]), but *Bolbostemma* and most species of *Gerrardanthus* have reticulate exines. Information on *Alsomitra macrocarpa* is ambiguous: its exine may be striate (Khunwasi, 1998) or reticulate (Marticorena, 1963). Cucurbitoideae pollen has a diameter of rarely up to >200 μm (some *Cayaponia* and *Polyclathra*; Khunwasi, 1998; Barth et al., 2005), a reticulate or echinate exine, and porate or colpate apertures.

Two tribes can be recognized by their pollen grains: Cucurbiteae (12/13) have echinate pollen that is 3-porate to stephanoporate; Sicyeae (Table 1, all genera investigated) have more finely spinulose pollen that is colpate or colpate. The remaining tribes, Coniandreae (including *Bambekea*, *Cucumeropsis*, and *Eureiandra*; 20/21) and Benincaseae (27/36), tend to have tricolporate pollen.

Praecitrullus and *Diplocyclos* of Benincaseae independently acquired echinate exines similar to those of Cucurbitaceae.

Another example of parallel evolution is provided by the pollen tetrads of *Gurania/Psiguria* in Coniandreae and *Borneosicyos* in Benincaseae, which clearly evolved independently (Fig. 1). Moreover, the nesting of *Helmontia* in *Gurania/Psiguria* (Fig. 1), if confirmed with denser species sampling, implies a reversal from pollen tetrads to monads. All three genera share two-staminate androecia, a rare state in the family (above), and their delimitations from each other have long been problematic (Jeffrey, 1962b, 1978); indeed “the taxonomic validity of Cogniaux’s segregate genus *Gurania* [...] as at present established is doubtful” (Jeffrey, 1962b).

In spite of these examples of parallel evolution of pollen characters, Cucurbitaceae are among those few families of flowering plants in which pollen carries phylogenetic signal at higher (tribal) levels (viz. Cucurbitaceae, Sicyeae). Among the earliest fossils of the family is the pollen *Hexacolpites echinatus* from the Oligocene of Cameroon (Salard-Cheboldaeff, 1978; Muller, 1985); these grains under the light microscope are hexacolpate or stephanocolpate and resemble polycolpate pollen of New World clade Sicyeae.

4.5. Chromosome numbers, ploidy levels, and genome evolution

Chromosome numbers are available for at least 141 species in 43 of the 130 genera (Beevy and Kuriachan, 1996; Index to Plant Chromosome Numbers, <http://mobot.mobot.org/W3T/Search/ipcn.html>), mostly those with important crop plants. Reported haploid chromosome numbers range from 7 to 24, with $x = 12$ a prevalent number (Beevy and Kuriachan, 1996).

In Nhandiroboideae, only *Gynostemma*, *Gomphogyne* (not sequenced), and *Hemsleya* have been studied cytologically. Numbers for species of *Gynostemma* are $n = 11, 22, 33, 44, 66, 88$ (Gao et al., 1995), *Gomphogyne* $n = 16$ (Thakur and Sinha, 1973), and *Hemsleya* $n = 14$ (Samuel et al., 1995). In Cucurbitoidaeae, chromosome numbers of genera in the basal grade (Fig. 1) range from $n = 9$ in *Thladiantha*, $n = 12$ in *Siraitia*, $n = 16$ in *Sinobaijiana* (Li et al., 1993, as *Baijiana*), to $n = 11$ or 14 in *Momordica* (Beevy and Kuriachan, 1996). The Bryonieae usually have $n = 10$ (S. Volz, personal communication, March 2007). The Schizopeponeae/Herpetospermeae have $n = 10$ in *Schizopepon* (Nishikawa, 1981) and $n = 11$ in *Edgaria* (Thakur and Sinha, 1973). In the LST clade, *Luffa* has $n = 13$ (Whitaker, 1933; Samuel et al., 1995), *Hodgsonia* $n = 9$ (Chen, 1993), *Trichosanthes* $n = 11$, *Gymnopetalum* $n = 12$ (Beevy and Kuriachan, 1996), and Sicyeae $n = 12$ (*Echinopepon*; Ward and Spellenberg, 1988) or $n = 16$ (*Cyclanthera*, *Echinocystis*; Samuel et al., 1995; Gervais et al., 1999). The few counted Coniandreae have $n = 13$ (*Corallocarpus*, *Kedrostis*; Beevy and Kuriachan, 1996) or $n = 14$ (*Apodanthera*; Ward, 1984). Benincaseae may have a base number of $n = 12$, as reported for eight of their genera (Kirkbride,

1993; Beevy and Kuriachan, 1996), but there is also much polyploidy and aneuploidy (Thakur and Sinha, 1973; Beevy and Kuriachan, 1996). Finally, Cucurbitaceae appear to have fixed polyploidy, with $n = 20$ (*Sicana*; Mercado and Lira, 1994; *Cucurbita*; Samuel et al., 1995).

Based on the available counts and the evolutionary directions implied by the phylogenetic tree, polyploidization appears to have occurred more often than previously suspected (Jeffrey, 1980). Ongoing work on Cucurbitaceae genomics must take into account the family’s unusually labile genome organization. Of the few known cases in the angiosperms of independent transfers of the *rbcL* gene from the plastome into the mitochondrial genome, two or three have occurred in the Cucurbitaceae (*Cucumis sativus*, *Cucurbita pepo*, *Cucurbita maxima* (Stern, 1987; Cummings et al., 2003). Our phylogeny implies that the transfer event in the *Cucurbita* lineage was independent from that in *Cucumis* (Fig. 1), which answers the question raised by Cummings et al. (2003), whether five or six independent chloroplast/mitochondrion *rbcL* transfers have occurred in the angiosperms. *Cucumis* has the largest known angiosperm mitochondria, with 1500 kb in *C. sativus* and 2400 kb in *C. melo* (Ward et al., 1981), and coincidentally the mitochondria of both species are paternally transmitted (Havey, 1997; Havey et al., 1998). The complete *C. sativus* chloroplast genome has been sequenced (Kim et al., 2005), and sequencing of several *Cucumis* mitochondrial genomes is ongoing. The phylogenetic framework for the family provided here will enable researchers to choose appropriate taxa for comparison and to infer the likely evolutionary direction of character change within the family.

4.6. Cucurbitaceae classification

The most recent subfamilial and tribal classification of Cucurbitaceae (Jeffrey, 2005; our Table 1) is largely supported by our chloroplast data, which recover eight of the 11 tribes almost exactly as circumscribed by Jeffrey. His Joliffieae and Trichosantheae, however, are poly- and paraphyletic (as discussed above) and will need to be abandoned, while his Benincaseae need only minor adjustments to attain monophyly (namely the removal of *Bambekea*, *Cogniauxia*, *Cucumeropsis*, *Eureiandra*, and *Nothoalsomitra*; cf. Fig. 1). In contrast to the tribes, few of Jeffrey’s 14 subtribes are monophyletic (Table 1 vs. Fig. 1). Many of these subtribes include only a few genera and could be abandoned without noticeable detrimental effects to the utility of the classification.

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