

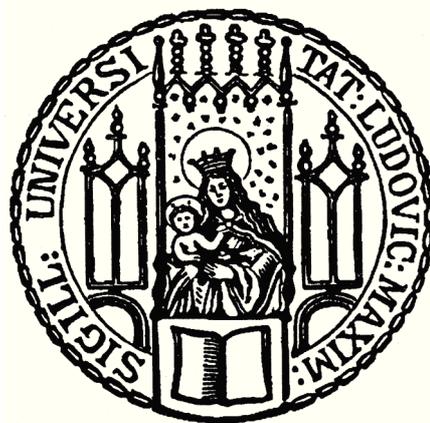
DISSERTATION  
ZUR ERLANGUNG DES DOKTORGRADES DER  
NATURWISSENSCHAFTEN  
AN DER FAKULTÄT FÜR BIOLOGIE DER  
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Phylogenetics and biogeography of two clades  
of Cucurbitaceae:  
one New World, one Old World, and both  
with economically important species

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München, September 2011



## **Erklärung**

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Susanne S. Renner betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist, und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

## **Ehrenwörtliche Versicherung**

Ich versichere hiermit ehrenwörtlich, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

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## Note

In this thesis, I present the results from my doctoral research, carried out under the guidance of Prof. Susanne S. Renner at the University of Munich (Ludwig-Maximilians-Universität München) from June 2008 until September 2011. The results from my research have contributed to five manuscripts that have been published in, or submitted to, international peer-reviewed journals. These are presented in the five chapters of this thesis. All of them have resulted from collaborations with other scientists, and my contributions to each of them were as follows:

**Chapter 1:** S. Renner and myself designed the research together. I generated, analyzed and interpreted the data. The manuscript was written by myself and S. Renner. All co-authors helped with interpretation of the data. Chapter 1 has been published in the *Proceedings of the National Academy of Sciences USA* (IF 2010: 9.771) under the following title:

**Sebastian, P.**, Schaefer, H., Telford, I.R.H. and Renner, S.S. (2010) Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences USA*, 107: 14269-14273.

**Chapter 2:** I. Telford and myself studied herbarium specimens and conducted fieldwork, I generated and analyzed the DNA data. Species descriptions and the key were prepared by I. Telford. The manuscript was written by I. Telford and myself, and revised by S. Renner. J. Bruhl prepared the distribution maps. The manuscript was published in *Systematic Botany* (IF 2010: 1.897) under the following title:

Telford, I.R.H., **Sebastian, P.**, Bruhl, J.J. and Renner, S.S. (2011) *Cucumis* (Cucurbitaceae) in Australia and eastern Malesia, including newly recognized species and the sister species to *C. melo*. *Systematic Botany*, 36: 376-389.

**Chapter 3:** Data for this chapter were generated and evaluated by myself, except for the sequences of Darwin's specimen of *S. villosus*, which were generated by H. Schaefer. The manuscript was written by all authors in equal parts and published in *Journal of Biogeography* (IF 2010: 4.273) under the title:

**Sebastian, P.**, Schaefer, H. and Renner, S.S. (2010) Darwin's Galapagos gourd: providing new insights 175 years after his visit. *Journal of Biogeography*, 35: 975-978.

**Chapter 4:** S. Renner and myself designed the research together. I generated, analyzed and interpreted the data. The manuscript was written by myself and S. Renner. The other co-authors contributed the majority of DNA samples and commented on the manuscript. The manuscript is currently in review for *Journal of Biogeography* (IF 2010: 4.273) under the title:

**Sebastian, P.**, Schaefer, H., Lira, R., Telford, I.R.H. and Renner, S.S. Radiation following long-distance dispersal: the contributions of time, opportunity, and diaspore morphology in *Sicyos* (Cucurbitaceae).

**Chapter 5:** For this chapter, I, Telford and myself conducted fieldwork together, and both of us studied herbarium specimens. Telford conducted the phenetic analyses with help from J. Bruhl and prepared the species descriptions and key to the species, while I generated, analyzed, and interpreted the molecular data. Both of us wrote the main text, and S. Renner revised the manuscript, which is currently in review for *Australian Systematic Botany* (IF 2010: 0.961) under the title:

Telford, I.R.H., **Sebastian, P.**, Bruhl, J.J. and Renner, S.S. *Sicyos* (Cucurbitaceae) in the south-western Pacific.

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## Summary

The gourd family, Cucurbitaceae, is among the economically most important families of plants, with many crop species that form the basis of multi-million dollar industries. Knowledge of these species' geographic origin and their closest wild relatives is fundamental to breeding efforts, genetic improvement, and conservation. Surprisingly, these aspects have been unknown or misunderstood for many widely cultivated species, even though plant material that could have been used for broad phylogenetic studies has long been available in herbaria. For the thesis presented here, I focused on the phylogenetic relationships within two clades of Cucurbitaceae that comprise cultivated species: the genus *Cucumis*, to which cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) belong, and the New World Sicyoeae, which contain vegetable pear or chayote (*Sicyos (Sechium) edulis*) and tacaco (*Frantzia tacaco*), locally important in Mexico and Costa Rica, and the former also cultivated worldwide. I used a combination of DNA sequence data from up to 175-year old herbarium specimens and molecular phylogenetic methods as well as traditional morphological and ecological data from my own fieldwork in Asia and Australia to infer the phylogenetic relationships among these clades. I also discovered and described several new species, and reconstructed plausible scenarios for the two clades' geographical unfolding over time.

Until recently, only two species of *Cucumis*, namely cucumber and its closest relative *C. hystrix*, were thought to be of Asian origin, and melon was thought to have originated in Africa, from where 30 species were known. Using DNA sequences from plastid and nuclear markers for some 100 *Cucumis* accessions from Africa, Australia, and Asia, I have shown that cucumber and melon both are of Asian (probably Indian) origin and form a clade with 23 previously overlooked species-level relatives in Asia, Australia, and around the Indian Ocean, at least nine of them new to science and some described as part of this thesis. Fieldwork I carried out in Thailand and Australia contributed new knowledge about the life forms and habitats of some of these species and resulted in fertile material essential for the descriptions. My study furthermore revealed that the sister species of melon is the re-discovered *C. picrocarpus* from Australia. Future breeding efforts and investigations of wild species related to melon and cucumber should therefore concentrate on Asia and Australia, instead of Africa.

In my second study group, the Sicyoeae, my aim was to test long-problematic generic boundaries and to reconstruct the history of the tribe's name-giving genus, *Sicyos*, which has an exceptional geographical distribution. Using a densely sampled molecular phylogeny that included type species of 23 currently or formerly accepted genera of Sicyoeae, I showed that morphology-based concepts did not result in monophyletic genera, and that species from

numerous smaller genera, including chayote, need to be part of *Sicyos* if monophyly is to be established. *Sicyos*, in its new circumscription, has a center of distribution in the Neotropics, where *c.* 50 species occur, but long-distance dispersal has resulted in the group's presence on Hawaii (where it radiated into 14 species), at least two arrivals on the Galápagos archipelago (but no radiations), and one arrival in Australia and New Zealand, now with three species, two of them new to science. Using molecular clock models, I dated these four trans-Pacific dispersal events, all from the American mainland, to the last 4.5 to 1 million years. The mode of dispersal may have been adherence of the small, spiny fruits to birds, which would fit with the documented occurrence of *Sicyos* plants near seabird nesting colonies. The rapid diversification on Hawaii may have followed the loss of the fruit spines in the ancestor of the 14 Hawaiian species, leading to lower dispersal ability and faster allopatric speciation in the diverse habitats of the archipelago.

## Zusammenfassung

Die Kürbisgewächse (Cucurbitaceae) beinhalten Nutzpflanzen, welche die Grundlage millionenschwerer Industriezweige bilden, und zählen zu den weltweit wirtschaftlich bedeutendsten Pflanzenfamilien. Es mag überraschend sein, dass bisher wenig über die Ursprungsgebiete und nächstverwandten Wildarten vieler dieser Kulturpflanzen bekannt oder untersucht worden ist, obwohl Pflanzenmaterial, das für weit angelegte phylogenetische Studien herangezogen werden müsste, seit langem in Herbarien zugänglich ist. Dieses Wissen ist wesentlich, nicht nur für genetische Verbesserung, z.B. in der Resistenz gegen Schädlinge, sondern auch um die wildwachsenden Verwandten von Kulturpflanzen erhalten zu können. Im Rahmen der vorliegenden Dissertation habe ich mich mit den phylogenetischen Verwandtschaftsverhältnissen innerhalb zweier Gruppen der Cucurbitaceae beschäftigt, die beide Nutzpflanzen enthalten. Dies waren die Gattung *Cucumis*, zu der die Gurke (*Cucumis sativus*) und die Melone (*Cucumis melo*) gehören, und die neuweltlichen Sicyoeae, zu denen die Chayote (*Sicyos (Sechium) edulis*) und die Tacaco (*Frantzia tacaco*) gehören, zwei lokal wichtige Gemüsearten aus Mexico und Costa Rica, wobei erstere auch in den altweltlichen Tropen angebaut wird. Ich habe molekular-phylogenetische Methoden zur Auswertung von DNA Sequenzdaten aus bis zu 175 Jahre alten Herbarbelegen, morphologische Studien und Feldarbeit in Asien und Australien benutzt, um die phylogenetischen Verwandtschaftsverhältnisse innerhalb dieser beiden Gruppen auf breiter Grundlage zu untersuchen. Meine Ergebnisse führten zur Entdeckung und Beschreibung neuer Arten und zu mehreren neuen Gattungsgrenzen. Des Weiteren habe ich für beide Gruppen, *Cucumis* und die Sicyoeae, mögliche Szenarien für deren geographische Entfaltung über die Zeit rekonstruiert.

Bis vor wenigen Jahren dachte man, dass nur zwei Arten der Gattung *Cucumis*, die Gurke und ihre Schwesterart *C. hystrix*, asiatischen Ursprungs seien. Die übrigen 30 *Cucumis* Arten stammten aus Afrika, und so ging man davon aus, dass auch die Melone afrikanischen Ursprungs sei. Durch die Auswertung von DNA Sequenzen aus den plastidären und nukleären Genomen von etwa 100 *Cucumis* Akzessionen aus Afrika, Australien und Asien konnte ich zeigen, dass sowohl Gurke als auch Melone asiatischen (wahrscheinlich indischen) Ursprungs und Teil einer Verwandtschaftsgruppe sind, die auch 23 weitere bisher falsch eingeordnete oder unbekannte Arten aus Asien, Australien und der Region um den Indischen Ozean umfasst. Einige der neun neu entdeckten Arten wurden im Zuge meiner Dissertation beschrieben. Meine Feldarbeit in Thailand und Australien erweiterte auch das Wissen über die Lebensformen und Habitate einiger dieser Arten und lieferte fertiles Pflanzenmaterial, das

für die Artbeschreibungen nötig war. Es zeigt sich auch, dass eine von mir wiederentdeckte *Cucumis*-Art aus Australien, *C. picrocarpus*, die nächstverwandte Wildart der Melone ist. Künftige Züchtungsprogramme und Nachforschungen über die wilden Verwandten von Gurke und Melone sollten sich daher auf die asiatischen und australischen anstatt wie bisher auf die afrikanischen *Cucumis* Arten konzentrieren.

In der zweiten von mir untersuchten Verwandtschaftsgruppe, den Sicyoeae, war es mein Anliegen, seit langem umstrittene Gattungsumschreibungen zu überprüfen und die geschichtliche Entfaltung der für den Tribus namensgebenden Gattung *Sicyos* zu rekonstruieren, da diese eine außergewöhnliche geographische Verbreitung aufweist. Anhand eines molekularen Stammbaums, der die Typusarten von 23 derzeit oder früher akzeptierten neuweltlichen Gattungen der Sicyoeae und die meisten ihrer bekannten Arten enthält, konnte gezeigt werden, dass die auf morphologischen Merkmalen basierenden Gattungskonzepte keine natürlichen Gruppen umschreiben, und dass Arten aus mehreren kleineren Gattungen, darunter auch die Chayote, in *Sicyos* eingegliedert werden müssen, damit diese zu einer monophyletischen Gattung wird. In der neuen Umschreibung umfasst *Sicyos* 50 Arten, die vor allem in der Neotropis vorkommen. Durch Fernverbreitung ist *Sicyos* jedoch auch in mehrere Regionen des Pazifiks gelangt. So gibt es auf Hawaii 14 Arten mit einem gemeinsamen Vorfahren, auf den Gálapagos-Inseln zwei Arten, die aber das Ergebnis zweier separater Besiedelungen sind und in der Region von Australien und Neuseeland drei Arten (mit einem gemeinsamen Vorfahren), von denen zwei im Zuge meiner Arbeit neu beschrieben wurden. Durch Anwendung einer molekularen Uhr konnte ich die vier trans-pazifischen Verbreitungsereignisse, die scheinbar alle vom amerikanischen Festland ausgingen, auf die letzten 4,5 bis 1 Millionen Jahre datieren. Die Verbreitung könnte durch ein Anhaften der kleinen, stacheligen Früchte an Vögeln zustande gekommen sein, was dazu passen würde, dass manche *Sicyos* Arten in der Nähe von Seevogelkolonien vorkommen. Die schnelle Diversifikation auf Hawaii könnte damit zusammenhängen, dass der gemeinsame Vorfahre der hawaiianischen Arten scheinbar diese Stacheln verloren hat, was die Verbreitungsfähigkeit eingeschränkt und somit eine schnellere allopatrische Artbildung in den sehr unterschiedlichen Habitaten innerhalb des Archipels begünstigt haben könnte.

## General Introduction

Over the last 18 years (Chase *et al.*, 1993), advances in molecular genetic techniques have revolutionized the study of relationships among organisms and our understanding of the evolutionary processes that led to the extant diversity of species on our planet. Entire genomes of economically or scientifically important species are becoming available for research more and more rapidly, and with decreasing cost and effort. It may come as a surprise therefore that the closest wild relatives of many widely cultivated and sometimes fully sequenced crop species are still unknown due to a lack of studies that would include large and unbiased sampling of plant material and multiple genes from the different plant genomes. The likely places of origin and domestication of many important crops are only just beginning to be resolved, with recent examples including cassava (Olsen & Schaal, 1999), pumpkin (Sanjur *et al.*, 2002), corn (Matsuoka *et al.*, 2002), potato (Spooner *et al.*, 2005), and rice (Londo *et al.*, 2006). Knowledge of the natural geographic origin of a crop and of its closest relatives is important for its genetic improvement and conservation.

The family Cucurbitaceae is one of the economically most important families of plants. Even so, the origins and phylogenetic relationships among clades containing watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*), loofah (*Luffa acutangula*), bitter melon (*Momordica charantia*), chayote (*Sicyos (Sechium) edulis*), ivy gourd (*Coccinia grandis*), snake gourd (*Trichosanthes cucumerina*), and creeping cucumber (*Melothria pendula*) until recently were unknown or misunderstood (seen with the hind-sight of molecular phylogenetic studies). For my doctoral research, I focused on two clades of Cucurbitaceae that comprise three of these crops: the genus *Cucumis*, to which cucumber (*Cucumis sativus*) and melon (*Cucumis melo*) belong, and the New World Sicyoeae, especially their name-giving genus *Sicyos*, which contains chayote (*Sicyos (Sechium) edulis*). I generated DNA sequence data from herbarium specimens and fresh material collected during fieldwork in Asia and Australia and used molecular phylogenetic methods as well as traditional morphological and ecological data to infer the phylogenetic relationships among these two clades. My work led to the discovery of several new species and some of them are described as part of this thesis. I also reconstructed likely scenarios for the two clades' geographical unfolding over time.

## Cucurbitaceae

Cucurbitaceae, commonly known as cucurbits or gourds, are a family of 95 genera in 15 tribes comprising 940 to 980 species that are essentially distributed in the tropical and

subtropical areas of the world, with hotspots of diversity in Southeast Asia, West Africa, Madagascar and Mexico (Schaefer & Renner, 2011a). Cultivars of many Cucurbitaceae species, such as cucumber (*C. sativus*), melon (*C. melo*), watermelon (*Citrullus lanatus*), pumpkin or squash (*Cucurbita spec.*), and zucchini (*C. pepo*), form the basis of multi-million dollar industries making Cucurbitaceae one of the economically most important families of plants. Agricultural cultivation of cucurbit crops occupies an estimated nine million hectares of land worldwide with a total yield of *c.* 184 million tons produce per year (<http://faostat.fao.org>). Recent family-wide molecular phylogenetic studies have resolved the major relationships among genera of Cucurbitaceae (Kocyan *et al.*, 2007) and the biogeographic history of the family (Schaefer *et al.*, 2009). The origin of Cucurbitaceae lies in Asia, where they originated in the Late Cretaceous, some 60 million years ago, with all five of the deepest divergences in the family dating to this time period (Schaefer *et al.*, 2009). Since then, long-distance dispersals (LDD) have played an essential role throughout the biogeographic history of the family, which underwent at least 43 successful LDD's, the majority of them between Asia and Africa, back to Asia, between Africa and South America or Madagascar, and from Asia to Australia (Schaefer *et al.*, 2009). Cucurbitaceae, in their extant distribution, contain many striking range disjunctions, such as those found in *Cayaponia*, *Kedrostis*, *Luffa*, *Muellerargia*, *Trichosanthes*, and *Sicyos* (Schaefer *et al.*, 2009; Duchon & Renner, 2010), reflecting the groups' ability to disperse over large distances.

Dispersal is mostly by animals, often birds. Typical bird fruits that are rather small, red, and fleshy berries containing relatively small seeds, are found in *Bryonia*, *Cucumis*, *Diplocyclos*, and *Zehneria* (Schaefer & Renner, 2011a). Other species are adapted to transport on animals, such as the small, single-seeded fruits of many species of *Sicyos*, which bear retrorsely barbed spines on their surface that easily attach to the plumage or fur of animals passing by. Dispersal by wind or water is less common but also found in *Luffa* and *Cayaponia*, where the fruit veins remain as a floating web (Ridley, 1930; Schaefer & Renner, 2011a), or in the large fleshy buoyant fruits of *Hodgsonia*, *Fevillea* and *Sicana* that also seem adapted for water dispersal (Schaefer & Renner, 2011a).

Cucurbitaceae are typically annual climbing or trailing herbaceous vines but a perennial, hemicyptophytic habit with rootstocks is also common. The only tree among species of the family is *Dendrosicyos socotranus*, an endemic to Socotra, and its stems can reach up to one meter in diameter (Olson, 2003). A morphological synapomorphy of most Cucurbitaceae are tendrils and which have been lost only a few times, as for example in *Cucumis messorius* or *Dendrosicyos socotranus*. Tendrils are modified shoots (Lassnig,

1997), and their morphology can serve as reliable character for differentiation within the family.

Pollination is predominantly by bees but pollination by other groups such as hawkmoths, sphingids, flies, butterflies, and other insects has been observed, and even bats (Duchen & Renner, 2010) and hummingbirds (Murawski & Gilbert, 1986) visit flowers of some Cucurbitaceae. Switches between monoecy and dioecy have occurred many times within Cucurbitaceae (Kocyan *et al.*, 2007; Volz & Renner, 2008; Schaefer & Renner, 2010), and from the distribution of monoecy and dioecy on the family phylogeny it appears that dioecy is the ancestral condition (Schaefer & Renner, 2011a). Cucurbitaceae also show various modes of sex expression, and especially two species of *Cucumis*, *C. melo* and *C. sativus*, have served as a primary model system of sex expression studies (Tanurdzic & Banks, 2004; Ming *et al.*, 2011). The study of XY sex determination is another scientific field in which Cucurbitaceae play important roles. Inheritance of sex in any organism was first genetically analysed in *Bryonia dioica* (Correns, 1903), and *Coccinia grandis* is one of very few angiosperms with dimorphic sex chromosomes (Bhaduri & Bose, 1947; Ming *et al.*, 2011). Cucurbitaceae furthermore are model plants for vascular biology research on long-distance signaling events, because xylem and phloem sap can be easily extracted from the long, non-woody vines (Lough *et al.*, 2006; Xoconostle-Cázares, 1999).

Cucurbitacins, the most characteristic chemicals of the group, are bitter triterpenes that are toxic to most organisms but, at the same time, can also attract some specialized herbivorous insects (Da Costa & Jones, 1971; Balkerna-Boomstra *et al.*, 2003). Cucurbitacins can be effective in slowing or stopping cell division, and they are therefore the subject of much research for applications in medicine, especially treatment of cancer (Thoenissen *et al.*, 2009; Lee *et al.*, 2010). Since numerous Cucurbitaceae species have been domesticated and are grown as vegetables forming basic ingredients for human diet, an important achievement of Cucurbitaceae cultivation was the reduction of high Cucurbitacin contents in the cultivars, besides improvements in crop yield, plant health, and the acquisition of various tastes and fruit shapes.

## *Cucumis*

The Cucurbitaceae genus *Cucumis* comprises two economically important species, *Cucumis melo*, the honey melon, and *Cucumis sativus*, the cucumber. These two species are among the most widely cultivated vegetable crops worldwide, and cucumber ranks among the top ten vegetables in world production (Chen *et al.*, 2004). Complete genomes of three *Cucumis sativus* breeding lines have become available recently: the Chinese fresh market type inbred line 9930 (Huang *et al.*, 2009), the Polish B10 line Borszczagowski (Woycicki *et al.*, 2011), and the North American pickling type cultivar Gy14 (Cavagnaro *et al.*, 2010). Cucumber was the 7<sup>th</sup> angiosperm to have its complete genome sequenced, and the melon genome also is about to be completed (Benjak *et al.*, 2010). Just like all other completely sequenced angiosperms, cucumber and melon are of great economic and/or scientific interest.

Numerous labs are working on the genetic improvement of cucumber and melon, using traditional as well as genomic approaches. Until 2007, it was thought that the genus *Cucumis* comprised 32 species and was closely related to the genus *Cucumella*, with eleven species (Kirkbride, 1993, 1994). As traditionally understood, *Cucumis* was essentially an African genus, and only the cucumber, *Cucumis sativus*, and its close relative *Cucumis hystrix* Chakr., were thought to occur naturally in India, China, Myanmar, and Thailand (Kirkbride, 1993; De Wilde & Duyfjes, 2007). The other economically important species, *Cucumis melo*, was thought to be native in Africa, as the majority of the known *Cucumis* diversity (Whitaker & Davis, 1996; Kirkbride, 1993; De Wilde & Duyfjes, 2007; Ghebretinsae *et al.*, 2007a). This classification indeed still formed the basis for much of the applied research on *Cucumis* (see Proceedings of the Cucurbitaceae EUCARPIA working group, Avignon, France, 21-24 May 2008, <http://www.inra.fr/cucurbitaceae> 2008).

Molecular sequence data testing this assumption with the necessary broad species sampling became available in 2007, and revealed that the genus, in its traditional circumscription, was not a natural (monophyletic) group (Kocyan *et al.*, 2007). Instead, it became clear that the genus *Cucumis*, as circumscribed by Kirkbride (1993), was paraphyletic, with no less than five other genera embedded in it (Kocyan *et al.*, 2007). This discovery resulted in an effort to reassess the wild relatives of *C. melo* and *C. sativus* (Renner & Schaefer, 2008; Renner *et al.*, 2007; Ghebretinsae *et al.*, 2007a). These studies confirmed that the Asian species *Cucumis sativus* and *Cucumis hystrix* are closely related to the poorly known Indian species *Dicaelospermum ritchiei* of the monotypic genus *Dicaelospermum* (now part of *Cucumis*). Moreover, it was discovered that species from Southeast Asia and tropical Australia that had traditionally been placed in the genus *Mukia* (De Wilde & Duyfjes,

2006) instead were closely related to *C. sativus*, *C. hystrix*, and *D. ritchiei*. Two new classifications of *Cucumis* formalized the name changes required to achieve a monophyletic *Cucumis* by transferring all species of the smaller genera *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* into *Cucumis* (Ghebretinsae *et al.*, 2007b; Schaefer, 2007). To add to these discoveries, a new species of *Cucumis* was described from Vietnam (De Wilde & Duyfjes, 2007). This species, *C. debilis*, was known from a single collection made in 1931 and housed in the Paris herbarium. Finally, there were five Australian as yet undescribed species that Telford, in his Cucurbitaceae treatment for the Flora of Australia (Telford, 1982), had provisionally placed within the genus *Mukia* as *Mukia* spec. A-E. Three of them were found to belong to a new genus close to the Eurasian *Bryonia* and *Ecballium* and subsequently named *Austrobryonia* (Schaefer *et al.*, 2008a), whereas one of the remaining two was placed in the genus *Cucumis* (Renner *et al.*, 2007). Thus, at the beginning of my doctoral research, in the summer of 2008, 14 Asian and Australian species were formally transferred or suspected to be close to *C. sativus* but most of them had only been collected a few times, some only once, and molecular phylogenetic relationships of most of them had never been tested using DNA sequence data.

Most species of *Cucumis* are climbers or trailers, except *C. messorius*, which is a small shrub, and most of them are monoecious perennials. Only five species from Africa are known to be dioecious (Thulin, 1991). However, the sexual systems and habits of many species, especially the newly discovered Asian and Australian ones, remain poorly known because species are under-collected and have not been studied in the field. Typical *Cucumis* habitats range from bushland to wooden grassland and disturbed areas in tropical and subtropical rainforests (Kirkbride, 1993; P. Sebastian, personal observation in Thailand and Australia). The flowers are orange-yellow and pollinated by bees; the fruits appear to be adapted to animal dispersal. Several species are used for their edible parts or in folk medicine and extracts may be effective in the treatment of diabetes (Chandrasekar *et al.*, 1989; Roman-Ramos *et al.*, 1995).

Cucumber is a crop with a narrow genetic base and it is susceptible to many diseases and insect damage (Whitaker & Davis, 1996). Therefore, breeders have a great interest in improving resistance, possibly through traits from wild relatives, and for this reason, various approaches for interspecific crosses have been tried during the past few decades to overcome genetic barriers. Making crosses is difficult because *C. sativus*, so far, is the only known species in the genus that has a haploid chromosome number of  $n = 7$ . Most other species of *Cucumis* have  $2n = 2x = 24$  chromosomes. No inter-species crosses involving *C. sativus* or *C.*

*melo* have ever been fully successful (Kirkbride, 1993). However, only crosses involving the African species of *Cucumis* or cucumber's putatively single Asian relative *C. hystrix* (Chen *et al.*, 1997) had been tried before 2007 since it was not known that *C. sativus* and *C. melo* have several close relatives in Asia and Australia. Knowledge of these species' geographic occurrence and biological traits will also be critical when searching for new sources of genes for *Cucumis* improvement. New species from Africa also continue to come to light (Thulin, 1991; Widrechner *et al.*, 2008), but it was the growing realization that the evolution of *C. sativus* occurred as part of a larger diversification of *Cucumis* in Southeast Asia and Australia that led to the studies presented in this thesis.

The *Cucumis* diversity outside of Africa, discovered mostly during my research, raised the question of the geographic origin of *C. melo*. Based on the impressive species richness of *Cucumis* in Africa compared to the two previously known species from Asia, and the fact that, just like *C. melo*, all African species of the genus have  $2n = 2x = 24$  chromosomes, modern authors have held that *C. melo* is of African origin. However, nineteenth-century taxonomists had suggested an Asian origin and domestication of melon (Naudin, 1859), which is in accordance with data pointing to a greatest genetic diversity in Indian and East Asian landraces of *C. melo* (Tanaka *et al.*, 2007, Dwivedi *et al.*, 2010). Earlier phylogenetic studies that tested these assumptions with molecular data (but with an Africa-biased sampling) yielded contradictory results. One study that only used nuclear ITS data inferred that the South African *C. sagittatus* was the sister species of *C. melo* (Ghebretinsae *et al.*, 2007a), while another recovered *C. melo* as sister to a small clade of five Asian and one Australian species (Garcia-Mas *et al.*, 2004; Renner *et al.*, 2007; Renner & Schaefer, 2008).

The objectives of this study therefore were to infer species relationships in *Cucumis* based on chloroplast and nuclear sequences, to sample all potential new Asian and Australian members of the genus including type material of all *Cucumis* species ever described and across the entire geographic range, to infer the biogeographic history of the genus, and to collect and study several of the poorly known wild relatives of cucumber and melon in their natural habitats. Results from my research on *Cucumis* are presented in the first two chapters of this thesis.

## ***Sicyos***

My second study group comprises the New World genera of the Cucurbitaceae tribe Sicyoeae, particularly the tribe's name-giving genus *Sicyos*. Sicyoeae comprises many other cucurbit species that are used for their edible parts, among them the chayote or vegetable pear (*Sicyos (Sechium) edulis*), which was most likely domesticated in Mexico and is now grown throughout the world in tropical and subtropical climates (Lira, 1996), and the more locally cultivated tacaco (*Frantzia tacaco*), which is most important in Costa Rica, where it is native. Morphology-based circumscriptions of the Neotropical Sicyoeae have been even more complicated than those of *Cucumis*. Finding a good circumscription based on a densely sampled phylogeny, including type material of all previously described genera thought to belong to the tribe, was therefore one of the goals of my study. However, it is also the exceptionally wide geographical distribution of the genus *Sicyos* that deserves special attention.

According to the most recent classification of Cucurbitaceae (Schaefer & Renner, 2011a, b), the traditionally Neotropical tribe Sicyoeae is expanded to hold the Asian tribe Trichosantheae, the former subtribe Luffineae (tropics of the Old and New world) and the monotypic Australian genus *Nothoalsomitra*, which is sister to the rest of the tribe. Sicyoeae thus comprise a total of twelve genera with together about 264 to 266 species (already accounting for the results of my studies (Chapters 3-5), which show that a monophyletic *Sicyos* must include the species previously placed in several other genera). Molecular phylogenetic studies identified the genus *Linnaeosicyos*, with a single species on Hispaniola, as sister to all other New World Sicyoeae, which are monophyletic (Schaefer *et al.*, 2008b; Schaefer *et al.*, 2009). Within the New World Sicyoeae, species of *Sicyos* appear morphologically similar to species previously assigned to *Anomalosicyos*, *Cladocarpa*, *Costarica*, *Microsechium*, *Parasicyos*, *Pterosicyos*, *Sarx*, *Sechiopsis*, *Sechium*, *Skottsbergiliana*, *Sicyocarya*, *Sicyocaulis*, and *Sicyosperma*. They had been segregated mostly based on combinations of fruit and floral characters. Finding morphological traits that unequivocally delimitate genera within the clade comprising *Sicyos* and its aforementioned relatives has proven difficult. This is why species have been transferred back and forth between up to three of these genera. The morphological diversity among the segregate genera of New World Sicyoeae has been the subject of a wide range of studies that tried to find stable circumscriptions for species in the clade based on macromorphological and palynological data (Lira, 1997a, b), and these have produced various hypotheses about the evolutionary

relationships among them. However, no comprehensive phylogenetic study sampling the entire diversity of the clade had been published before.

*Sicyos* has a center of diversity in Mexico, where new species keep being discovered (Lira, 1994; Lira & Rodríguez-Arevalo, 1999; Rodríguez-Arevalo & Lira, 2001; Rodríguez-Arevalo, 2003; Rodríguez-Arevalo *et al.*, 2004, 2005), and, depending on its taxonomic circumscription, the genus includes a total of 41 or 50 species that range from Canada to Argentina. In addition to the species that are distributed throughout the Americas, *Sicyos* has managed to establish populations in four regions of the Pacific that must have been reached by long-distance dispersal (LDD). This has led to the presence of 14 endemic species of *Sicyos* on the Hawaiian Islands (Wagner & Shannon, 1999), two on the Galápagos Islands, two in Australia, and one in New Zealand (Chapter 4). Of the latter three, two species were identified as new to science by my molecular phylogeny and fieldwork in Australia (Chapter 5).

Even though the Hawaiian Islands are more than 3,800 km away from the nearest continent, there is abundant evidence for a strong American contribution to the Hawaiian flora (Baldwin & Wagner, 2010), and with the center of diversity in the Neotropics, the ancestor of the Hawaiian species most likely had arrived from there. The less isolated Galápagos archipelago (930 km from the closest mainland, Ecuador) was colonized twice (Chapters 3 and 4). In the Galápagos, the majority of the endemic plants are thought to be of western South American origin (Porter, 1984a, b), where several species of *Sicyos* also occur. However, judging from the extant species numbers, neither arrival led to a radiation event, and today, the two species of *Sicyos* on the Galápagos Islands are highly endangered or thought to be extinct. Possible reasons for their demise are discussed in Chapter 3.

Long-distance dispersal events from North, Central, or South America to the Hawaiian or the Galápagos Islands are relatively common, and the floras of both of these archipelagos exhibit high affinities to the American continents. In contrast, very few LDD's are known to have occurred across the Pacific from the Americas to Australia or New Zealand, one example being a clade of *Lepidium* (Brassicaceae) with Californian and Australian/New Zealand species, inferred to be the result of trans-Pacific long-distance dispersal (Mummenhoff *et al.*, 2004). *Sicyos* is today absent from other Pacific archipelagos that could have served as stepping-stones, except for Hawaii and the Galápagos. The only other Pacific islands where *Sicyos* has been collected are the Islas Desventuradas off the Chilean coast, Lord Howe Island (now extinct) and Norfolk Island in the Tasman Sea between Australia and New Zealand, the Kermadec Islands *c.* 1000 km northeast of New Zealand, and islands

adjacent to New Zealand's North Island. Thus, the most enthralling question concerning trans-Pacific dispersal in *Sicyos* was that of the geographic origin of the ancestor(s) of the Australian and New Zealand species.

The modes or mechanisms of dispersal over such long distances are difficult to infer (Nathan, 2006), but certain fruit traits are adaptations to dispersal by wind, rafting, floating, or movement by birds (Campbell, 1919; Carlquist, 1996; Cain *et al.*, 2000). The small (*c.* 5-10 mm long), one seeded fruits of *Sicyos* bear hooks on their surface, and with these they are able to adhere to birds. In this way they can be transported across the birds' migration routes. *Sicyos* can be found in various habitats from coastal areas and lowland shrublands to evergreen rainforests and montane cloud forests. Birds are thought to have played the most important role in plant colonization of the Hawaiian and Galápagos Islands (Porter, 1976; Wagner *et al.*, 1990). Storm petrels, albatros species, frigate birds, and other large sea birds are known to migrate between the Central- and South American coast and Pacific archipelagoes like the Hawaiian or the Galápagos Islands. But there have also been migrations recorded for these birds from Peru to New Zealand (Tomkins, 1982).

Given the above-described problems with the circumscription of *Sicyos*, and its interesting disjunct distribution on islands and continents thousands of kilometers apart from each other, my aim was to find a more natural circumscription of *Sicyos* and the other genera of New World Sicyoeae (which required comprehensive taxon sampling), and to infer the biogeographic history of *Sicyos* using inference of divergence times through molecular clock dating and reconstruction of ancestral areas, traits, and habitats.

## References

- Baldwin, B.G. & Wagner, W.L. (2010) Hawaiian angiosperm radiations of North American origin. *Annals of Botany*, **105**, 849–879.
- Balkerna-Boomstra, A.G., Zijlstra, S., Verstappen, F.W.A., Inggamer, H., Mercke, P.E., Jongsma, M.A. & Bouwmeester, H.J. (2003) Role of Cucurbitacin C in resistance to spider mite *Tetranychus urticae* in cucumber (*Cucumis sativus* L.). *Journal of Chemical Ecology*, **29**, 225-235.
- Benjak, A., Gonzalez, V., Mir, G., Arús, P., Aranda, M., Álvarez-Tejado, M., Droege, M., Du, L., Puigdomènech, P. & Garcia-Mas, J. (2010) A draft sequence of the melon genome revealed by high throughput shotgun sequencing. Plant & Animal Genomes XVIII Conference. Abstracts. Available at [http://www.intl-pag.org/18/abstracts/P01\\_PAG\\_XVIII\\_053.html](http://www.intl-pag.org/18/abstracts/P01_PAG_XVIII_053.html) (last accessed July 2011).
- Bhaduri, P.N. & Bose, P.C. (1947) Cyto-genetical investigations in some common cucurbits with special reference to fragmentation of chromosomes as a physical basis of speciation. *Journal of Genetics*, **48**, 237-256.
- Cain M.L., Milligan, B.G. & Strand, A.G. (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany*, **87**, 1217-1227.
- Campbell, D.H. (1919) *The derivation of the flora of Hawaii*, Stanford University Press,

Stanford, California.

- Carlquist, S. (1996) Plant dispersal and the origin of Pacific island floras. *The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns and processes* (ed. by A. Jeast and S.E. Miller), pp. 153-164. SPB Academic Publishing, Amsterdam, the Netherlands.
- Cavagnaro, P.F., Senalik, D.A., Yang, L., Simon, P.W., Harkins, T.T., Kodira, C.D., Huang, S. & Weng, Y. (2010) Genome-wide characterization of simple sequence repeats in cucumber (*Cucumis sativus* L.). *BMC Genomics*, **11**, 569, DOI: 10.1186/1471-2164-11-569.
- Chandrasekar, B., Mukherjee, B. & Mukherjee, S.K. (1989) Blood sugar lowering potentiality of selected Cucurbitaceae plants of Indian origin. *Indian Journal of Medical Research*, **90**, 300-305.
- Chase, M.W., Soltis, D.E., Olmstead, R.G. et al. (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden*, **40**, 528-580.
- Chen, J.-F., Staub, J.E., Tashiro, Y., Isshiki, S. & Miyazaki, S. (1997) Successful interspecific hybridization between *Cucumis sativus* L. and *C. hystrix* Chakr. *Euphytica*, **96**, 413-419.
- Chen, J.F., Zhuang, F.Y., Liu, X.A. & Qian, C.T. (2004) Reciprocal differences of morphological and DNA characters in interspecific hybridization in *Cucumis*. *Canadian Journal of Botany*, **82**, 16-21.
- Correns, C. (1903) Über die dominierenden Merkmale der Bastarde. *Berichte der Deutschen Botanischen Gesellschaft*, **21**, 133-147.
- Da Costa, C.P., & Jones, C.M. (1971) Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* L. *Science*, **172**, 1145-1146.
- De Wilde, W.J.J.O & Duyfjes, B.E.E. (2006) *Mukia* Arn. (Cucurbitaceae) in Asia, in particular in Thailand. *Thai Forest Bulletin (Bot.)*, **34**, 38-52.
- De Wilde, W. J. J. O. & Duyfjes, B.E.E. (2007) The wild species of *Cucumis* L. (Cucurbitaceae) in South-East Asia. *Adansonia*, sér. 3, **29**, 239-248.
- Duchen, P. & Renner, S.S. (2010) The evolution of *Cayaponia* (Cucurbitaceae): Repeated shifts from bat to bee pollination and long-distance dispersal to Africa 2-5 million years ago. *American Journal of Botany*, **97**, 1129-1141.
- Dwivedi, N.K., Dhariwal, O.P., Krishnan, S.G. & Bhandari, D.C. (2010) Distribution and extent of diversity in *Cucumis* species in the Aravalli ranges of India. *Genetic Resources and Crop Evolution*, **57**, 443-452.
- Garcia-Mas, J., Monforte, A.J. & Arús, P. (2004) Phylogenetic relationships among *Cucumis* species based on the ribosomal internal transcribed spacer sequence and microsatellite marker. *Plant Systematics and Evolution*, **248**, 191-203.
- Ghebretinsae, A.G., Thulin, M. & Barber, J.C. (2007a) Relationships of cucumbers and melons unraveled: Molecular phylogenetics of *Cucumis* and related genera (Benincaseae, Cucurbitaceae). *American Journal of Botany*, **94**, 1256-1266.
- Ghebretinsae, A.G., Thulin, M. & Barber, J.C. (2007b) Nomenclatural changes in *Cucumis* (Cucurbitaceae). *Novon*, **17**, 176-178.
- Huang, S., Ruiquiang, L., Zhang, Z., et al. (2009) The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics*, **41**, 1275-1281.
- Kirkbride, J.H., Jr. (1993) Biosystematic monograph of the genus *Cucumis* (Cucurbitaceae). Parkway Publishers, Boone, North Carolina.
- Kirkbride, J.H., Jr. (1994) Revision of *Cucumella* (Cucurbitaceae, Cucurbitoideae, Melothrieae, Cucumerinae). *Brittonia*, **46**, 161-186.
- Kocyan, A., Zhang, L.-B., Schaefer, H. & Renner, S.S. (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553-577.

- Lassnig, P. (1997) Verzweigungsmuster und Rankenbau der Cucurbitaceae. Tropische und subtropische Pflanzenwelt (Akademie Mainz). Verlag Franz Steiner, Stuttgart.
- Lee, D.H., Iwanski, G.B. & Thoennissen, N.H. (2010) Cucurbitacin: Ancient Compound Shedding New Light on Cancer Treatment. *The Scientific World Journal*, **10**, 413-418.
- Lira, R. (1994) Especie nueva de *Microsechium* (Cucurbitaceae, tribu Sicyoeae, subtribu Sicyinae) del estado de Oaxaca, México. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Serie Botánica*, **65**, 73-81.
- Lira, R. (1996) Chayote, *Sechium edule* (Jacq.) Sw. Institute of Plant Genetics and Crop Plant Research/International Plant Genetic Resources Institute, Rome, Italy.
- Lira, R., Caballero, J. & Dávila, P. (1997a) A contribution to the generic delimitation of *Sechium* (Cucurbitaceae, Sicyinae). *Taxon*, **46**, 269-282.
- Lira, R., Villasenor, J.L. & Dávila, P.D. (1997b) A cladistic analysis of the subtribe Sicyinae (Cucurbitaceae). *Systematic Botany*, **22**, 415-425.
- Lira R. & Rodríguez-Arévalo, I. (1999) Nuevas especies de los géneros *Cyclanthera* Schrad. y *Sicyos* L. (Sicyeae, Cucurbitaceae) para la flora de México. *Acta Botanica Mexicana*, **48**, 11-19.
- Londo, J.P., Chiang, Y.C., Hung, K.H., Chiang, T.Y. & Schaal, B.A. (2006) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. *Proceedings of the National Academy of Sciences USA*, **103**, 9578-9583.
- Lough, T.J. & Luca, W.J. (2006) Integrative plant biology: role of phloem long-distance macromolecular trafficking. *Annual Review of Plant Biology*, **57**, 203-232.
- Matsuoka, Y., Vigouroux, Y., Goodman, M.M., Sanchez, J., Buckler, E. & Doebley, J. (2002) A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences USA*, **99**, 6080-6084.
- Ming, R., Bendahmane, A. & Renner, S.S. (2011) Sex chromosomes in land plants. *Annual Review of Plant Biology*, **62**, 485-514.
- Mummenhoff, K., Linder, P., Friesen, N., Bowman, J.L., Lee, J-Y. & Franzke, A. (2004) Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. *American Journal of Botany*, **91**, 254-261.
- Murawski, D.A. & Gilbert, L.E. (1986) Pollen flow in *Psiguria warscewiczii*: a comparison of *Heliconius* butterflies and hummingbirds. *Oecologia*, **2**, 161-167.
- Nathan R. (2006) Long-distance dispersal of plants. *Science*, **313**, 786-788.
- Naudin, C. (1859) Essais d'une monographie des especès et des variétés du genre Cucumis. *Annales des Sciences Naturelles, Botanique* **4**, 5-87.
- Olsen, K.M. & Schaal, B.A. (1999) Evidence on the origin of cassava: Phylogeography of *Manihot esculenta*. *Proceedings of the National Academy of Sciences USA*, **96**, 5586-5591
- Olson M.E. (2003) Stem and leaf anatomy of the arborescent Cucurbitaceae *Dendrosicyos socotrana* with comments on the evolution of pachycauls from lianas. *Plant Systematics and Evolution*, **239**, 199-214.
- Porter D.M. (1976) Geography and dispersal of Galapagos Islands vascular plants. *Nature*, **264**, 745-746.
- Porter D.M. (1984a) Relationships of the Galapagos flora. *Biological Journal of the Linnean Society*, **21**, 243-251.
- Porter D.M. (1984b) Endemism and evolution in terrestrial plants. Key environments. Galapagos (ed. by R. Perry), pp. 85-99. Pergamon Press, Oxford.
- Renner, S.S., Schaefer, H. & Kocyan, A. (2007) Phylogenetics of *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evolutionary Biology*, **7**, 58.

- Renner, S.S. & Schaefer, H. (2008) Phylogenetics of *Cucumis* (Cucurbitaceae) as understood in 2008. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae* (ed. by M. Pitrat), pp 53-58, INRA, Avignon.
- Ridley, H.N. (1930) *The dispersal of plants throughout the world*. L. Reeve, Ashford.
- Rodríguez-Arévalo, I. & Lira, R. (2001) Nueva especie del género *Sicyos* L. (Cucurbitaceae) para la República Mexicana. *Boletín de la Sociedad Botánica de México*, **68**, 81–84.
- Rodríguez-Arévalo I. (2003) A new species of *Sicyos* (Cucurbitaceae, Sicyeae, Sicyinae) from Mexico and Guatemala. *Brittonia*, **55**, 69-72.
- Rodríguez-Arévalo I., Lira, R. & Dávila, P. (2004) Two new species of *Sicyos* L. (Cucurbitaceae) from Guerrero and Oaxaca, Mexico. *Botanical Journal of the Linnean Society*, **145**, 373-378.
- Rodríguez-Arévalo I., Lira, R. & Calzada, I. (2005) A new species of *Sicyos* L. (Cucurbitaceae) from Oaxaca, Mexico. *Brittonia*, **57**, 43-46.
- Roman-Ramos, R., Flores-Saenz, J.L. & Alarcon-Aguilar, F.J. (1995) Anti-hyperglycemic effect of some edible plants. *Journal of Ethnopharmacology*, **48**, 25-32.
- Sanjur, O.I., Piperno, D.R., Andres, C.T. & Wessel-Beaver, L. (2002) Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from a mitochondrial gene: Implications for crop plant evolution and areas of origin. *Proceedings of the National Academy of Sciences USA*, **99**, 10923-10928.
- Schaefer H. (2007) *Cucumis* (Cucurbitaceae) must include *Cucumella*, *Dicoelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce*: a recircumscription based on nuclear and plastid DNA data. *Blumea*, **52**, 165-177.
- Schaefer, H. & Renner, S.S. (2010) A three-genome phylogeny of *Momordica* (Cucurbitaceae) suggests seven returns from dioecy to monoecy and recent long distance dispersal to Asia. *Molecular Phylogenetics and Evolution*, **54**, 553-560.
- Schaefer, H. & Renner, S.S. (2011a) Cucurbitaceae. *Families and Genera of Vascular Plants* vol. 10. (ed. by K. Kubitzki), pp. 112–174. Springer Verlag, Berlin.
- Schaefer, H. & Renner, S.S. (2011b) Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon*, **60**, 122–138.
- Schaefer, H., Telford, I.R.H., & Renner, S.S. (2008a) *Austrobryonia* (Cucurbitaceae), a new Australian endemic genus, is the closest living relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*. *Systematic Botany*, **33**, 125-132.
- Schaefer H., Kocyan, A. & Renner, S.S. (2008b) *Linnaeosicyos* (Cucurbitaceae): a new genus for *Trichosanthes amara*, the Caribbean sister species of all Sicyeae. *Systematic Botany*, **33**, 349-355.
- Schaefer H., Heibl, C. & Renner, S.S. (2009) Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 843–851.
- Spooner, D.M., McLean, K., Ramsay, G., Waugh, R. & Bryan G.J. (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences USA*, **102**, 14694-14699.
- Tanaka, K., Nishitani, A., Akashi, Y., Sakata, Y., Nishida, H., Yoshino, H. & Kenji, K. (2007) Molecular characterization of South and East Asian melon, *Cucumis melo* L. and the origin of group *Conomon* var. *makuwa* and var. *conomon* revealed by RAPD analysis. *Euphytica*, **153**, 233-247.
- Tanurdzic, M. & Banks, J.A. (2004) Sex-Determining Mechanisms in Land Plants. *Plant Cell*, **16**, 61-71.
- Telford, I. R. (1982) Cucurbitaceae. *Flora of Australia* vol. 8 (ed. by A.S. George), pp. 158–198, Australian Government Publishing Service, Canberra.
- Thoennissen, N.H., Iwanski, G.B., Doan, N.B., Okamoto, R., Lin, P., Abbassi, S., Song, J.H., Yin, D., Toh, M., Xie, W.D., Said, J.W., & Koeffler, H.P. (2009) Cucurbitacin B

- induces apoptosis by inhibition of the JAS/STAT pathway and potentiates antiproliferative effects of Gemcitabine on pancreatic cancer cells. *Cancer Research*, **69**, 5876.
- Thulin M. (1991) New species of *Cucumis* (Cucurbitaceae) from northeast tropical Africa. *Nordic Journal of Botany*, **11**, 535-542.
- Tomkins, R.J. (1982) Migration of White-faced Storm-Petrels *Pelagodroma marina* in the South Pacific and the status of the Kermadec subspecies. *Emu*, **84**, 32–35.
- Volz, S.M. & Renner, S.S. (2008) Hybridization, polyploidy, and evolutionary transitions between monoecy and dioecy in *Bryonia* (Cucurbitaceae). *American Journal of Botany*, **95**, 1297-1306.
- Wagner, W.L. & Shannon, R.K. (1999) Nomenclator of Hawaiian *Sicyos* (Cucurbitaceae). *Novon*, **9**, 441–447.
- Wagner, W.L., Herbst D.R. & Sohmer S.H. (1990) *Manual of the Flowering Plants of Hawai'i*. University of Hawaii Press & Bishop Museum Press, Honolulu.
- Whitaker, T.W., & Davis, G.N. (1996) *Cucurbits – Botany, Cultivation, Utilization*. Interscience Publishers, New York.
- Widrechner, M.P., Kirkbride, J.H. Jr., Ghebretinsae, A.G. & Reitsma, K.R. (2008) *Cucumis zambianus* (Cucurbitaceae), a New Species from Northwestern Zambia. *Systematic Botany*, **33**, 732–738.
- Woycicki, R., Gutman, J., Gawronski, P., *et al.* (2011) The genome sequence of the North-European cucumber (*Cucumis sativus* L.) unravels evolutionary adaptation mechanisms in plants. *PLOS ONE*, **6**: e22728.
- Xoconostle-Cázares, B., Xiang, Y., Ruiz-Medrano, R., Wang, H.-L., Monzer, J., Yoo, B.-C., McFarland, K.C., Franceschi, V.R. & Lucas, W.J. (1999) Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science*, **283**, 94-98.



## CHAPTER 1

CUCUMBER (*CUCUMIS SATIVUS*) AND MELON (*C. MELO*) HAVE  
NUMEROUS WILD RELATIVES IN ASIA AND AUSTRALIA, AND THE  
SISTER SPECIES OF MELON IS FROM AUSTRALIA

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# Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia

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Among the fundamental questions regarding cultivated plants is their geographic origin and region of domestication. The genus *Cucumis*, which includes cucumber (*Cucumis sativus*) and melon (*Cucumis melo*), has numerous wild African species, and it has therefore been assumed that melon originated in Africa. For cucumber, this seemed less likely because wild cucumbers exist in India and a closely related species lives in the Eastern Himalayas. Using DNA sequences from plastid and nuclear markers for some 100 *Cucumis* accessions from Africa, Australia, and Asia, we show here that melon and cucumber are of Asian origin and have numerous previously overlooked species-level relatives in Australia and around the Indian Ocean. The wild progenitor of *C. melo* occurs in India, and our data confirm that the Southeast Asian *Cucumis hystrix* is the closest relative of cucumber. Most surprisingly, the closest relative of melon is *Cucumis picocarpus* from Australia. *C. melo* diverged from this Australian sister species approximately 3 Ma, and both diverged from the remaining Asian/Australian species approximately 10 Ma. The Asian/Australian *Cucumis* clade comprises at least 25 species, nine of them new to science, and diverged from its African relatives in the Miocene, approximately 12 Ma. Range reconstruction under maximum likelihood suggests Asia as the ancestral area for the most recent common ancestor of melon and cucumber, fitting with both having progenitor populations in the Himalayan region and high genetic diversity of *C. melo* landraces in India and China. Future investigations of wild species related to melon and cucumber should concentrate on Asia and Australia.

ancestral areas | crops | economic plants | wild progenitors

Among the most fundamental and debated questions regarding the evolution of cultivated plants is their geographic origin and region of domestication (1). Recent phylogeographic and phylogenetic work on cassava, pumpkin, corn, potato, and rice (2–6) has uncovered the likely places of origin and domestication of these crops. Although many premolecular hypotheses about the domestication of particular species still require testing, it is clear that the Indo-Chinese region has produced a particularly long list of crops. These include rice (*Oryza sativa*), millets (*Setaria* spp.), beans (*Vigna mungo*; *Vigna radiata*), angled loofah (*Luffa acutangula*), yams (*Dioscorea* spp.), and taro (*Colocasia esculenta*) (7–9). Archaeological evidence from northern India documents these Neolithic crops from 7,000 BC onward, and by the early second millennium, there is evidence of Western crops arriving in India through trade, such as wheat, barley, lentils, grasspea, and peas (7).

One of the crops domesticated in the Indo-Gangetic plain is cucumber, *Cucumis sativus*. Evidence for this consists in the occurrence there of a wild progenitor, *C. sativus* var. *hardwickii* (10, 11) and in comparative linguistic evidence (7, 8). Fossil seeds of cucumber and melon cannot be reliably distinguished, and archeological reports therefore are of limited value for pinpointing areas of melon or cucumber domestication or identifying the routes by which these crops arrived in a particular region (12). Cucumber and melon today are among the 20 most important vegetable crops worldwide

(13). The first complete genome of cucumber was released last year (14), and the genome of melon is being completed (15).

In contrast to cucumber, the geographic origin and region of domestication of melon (*Cucumis melo*) have remained unclear. Nineteenth-century taxonomists suggested that melon probably originated and was domesticated in Asia (e.g., ref. 11). This idea became discredited as workers began to study *Cucumis* chromosome numbers (16). *C. sativus* has a chromosome number of  $2n = 14$ , whereas *C. melo* has a chromosome number of  $2n = 24$ . At least 30 other species of *Cucumis* have had their chromosomes counted, all but one from Africa and all having  $2n = 24$  or multiples thereof (17). Based on the impressive species richness in Africa and the identical chromosome number of *C. melo* and African *Cucumis*, modern authors have held that *C. melo* is of African origin (18–22). This view has persisted even in the face of genetic data pointing to greatest genetic diversity in Indian and East Asian landraces of *C. melo* (23–26) and despite numerous failed attempts to produce fertile F1 offspring from crosses of *C. melo* and African species of *Cucumis* (17).

Phylogenetic studies on the genus *Cucumis* have been Africa-biased in terms of the included species (27–29). These studies yielded contradictory results regarding the closest relatives of *C. sativus* and *C. melo*. A 2007 study (29) inferred that South African *C. sagittatus* is the sister species of *C. melo*, although this was only observed with nuclear ribosomal internal transcribed spacer (ITS) sequences, not chloroplast sequences. Other studies that also used ITS (27, 28, 30) found *C. melo* isolated from the other included African species and instead sister to a small clade of five Asian and one Australian species. Although not resolving the position of *C. melo*, these studies clarified that the genus *Cucumis* in its traditional circumscription (17) was paraphyletic (28, 29, 31), with species from five other genera nested inside it. [The necessary nomenclatural changes were made previously (32, 33); here we use these up-dated species names.]

Here we investigate the evolutionary relationships and geographic origin of melon and cucumber by sampling *Cucumis* accessions covering the entire natural range of the genus from Africa to Southeast Asia to Australia, the Pacific and the Indian Ocean islands. The geographic origin of the plants sequenced is shown in Fig. 1. We included new collections from fieldwork in Australia and Thailand as well as old material from herbaria, including type material of long synonymized names.

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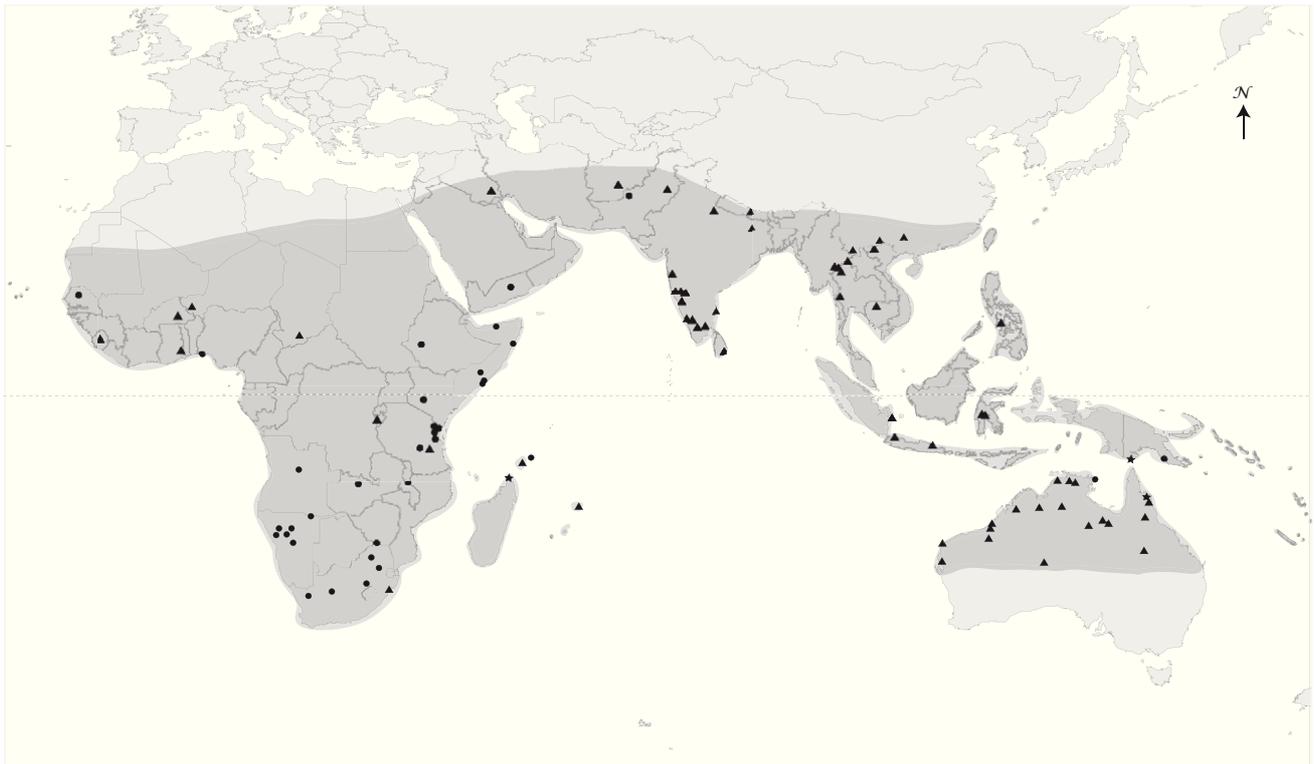
The authors declare no conflict of interest.

\*This Direct Submission article had a prearranged editor.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database. For a list of accession numbers, see Table S1.

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**Fig. 1.** The natural geographic range of *Cucumis* (shaded) and the geographic origin of the sequenced plant material. Triangles, Asian/Australian clade comprising cucumber and melon; circles, African grade; stars, outgroup.

Knowing the progenitors and closest relatives of melon and cucumber is important because these crops are highly susceptible to drought and pathogens, including powdery mildew and several mosaic viruses (14). Besides the interest in varieties with new taste or fruit shape, there is great interest in introducing resistance genes from wild relatives. In the case of melon, the search for these relatives has so far concentrated on Africa because of the view that *C. melo* is of African origin (18–22).

## Results

**Closest Relatives of Cucumber and Melon.** A maximum likelihood phylogeny from the combined plastid and nuclear data (Fig. 2) shows that *C. sativus* is sister to *Cucumis hystrix*, confirming previous findings (28–30). *Cucumis setosus*, an entity that the most recent monograph of *Cucumis* (17) synonymized under *C. sativus*, instead is closest to *Cucumis silentvalleyi* and *Cucumis indicus* from southern and southwestern India. Sequences of the Himalayan entities *Cucumis trigonus* and *Cucumis callosus* are nearly identical to those of *C. melo* (Fig. 2) and likely represent the wild progenitor of cultivated melon as these forms are fully crossable with *C. melo* (34). The sister species to *C. melo* populations is the Australian *Cucumis picrocarpus*, which had been synonymized under *C. melo* (17), but is genetically and morphologically highly distinct (Fig. 2; Fig. 3 shows a color photo of *C. picrocarpus*).

Cucumber and melon are part of a clade that comprises 25 poorly collected and understudied species-level entities from India, Indochina, Malesia, Australia, Africa, and Indian Ocean islands (Figs. 1 and 2). Species recently transferred to *Cucumis* based on morphology (32, 33) indeed belong in the genus as indicated by chloroplast and nuclear DNA sequences (Fig. 2). Our results, however, reveal another 18 close relatives (labeled in Fig. 2) of cucumber and melon that were neither accepted in the 1993 monograph of the genus by Kirkbride (17) nor included in previous phylogenetic studies (29, 30). Nine (labeled with an upper script N in Fig. 2) represent as yet undescribed species. An important factor in the unexpected Asian/

Australian diversity of *Cucumis* is *Cucumis* (*Mukia*) “*maderaspatanus*.” This polymorphic taxon, revised in 2006 (35), turns out to be a highly unnatural assembly comprising at least nine species-level entities (labeled with an upper script M in Fig. 2) that are not close to each other (Fig. 2 and Table S1). *Cucumis maderaspatanus* was thought to range from Africa across Asia to Australia, but accessions from these three continents do not group together.

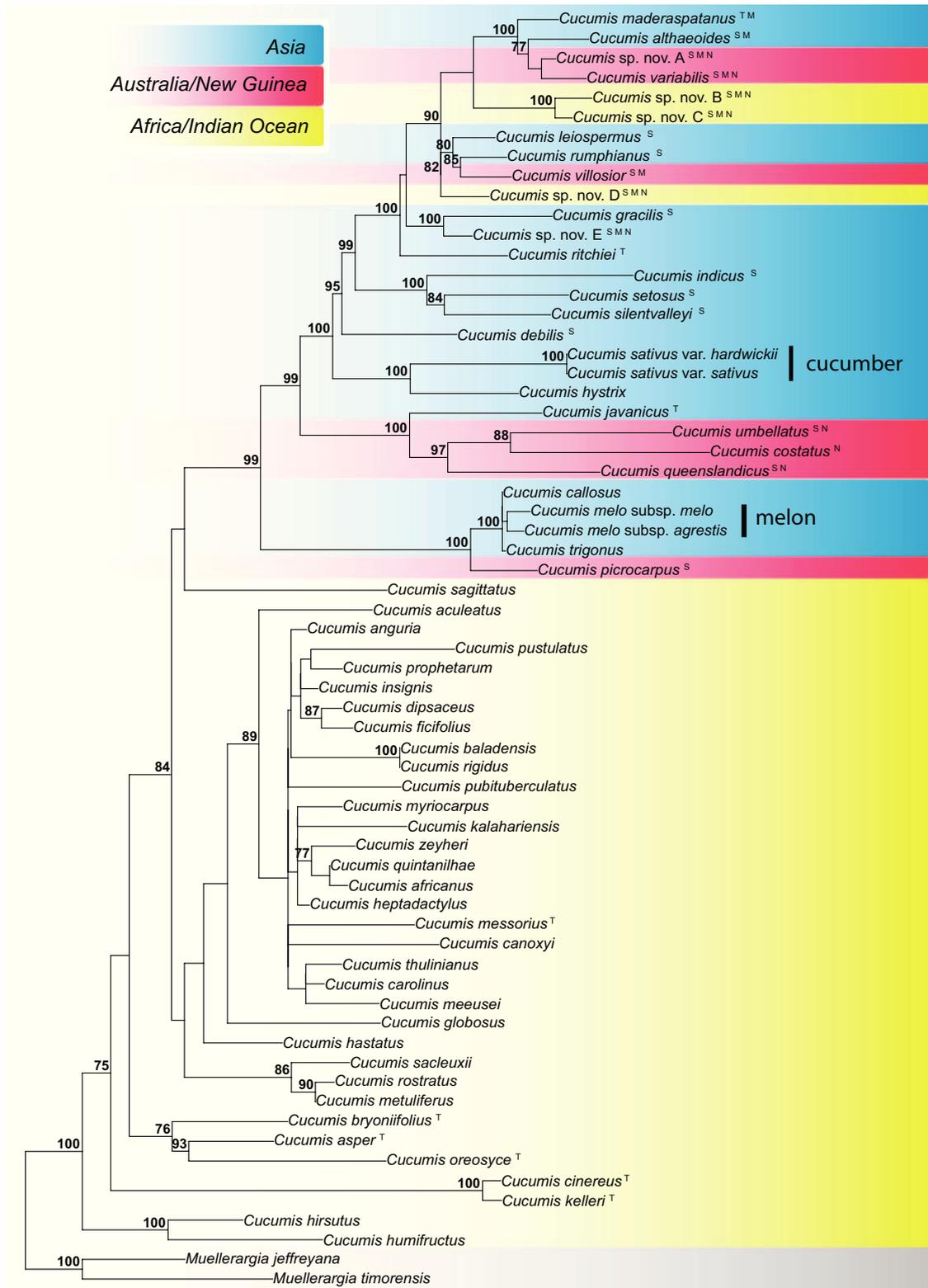
Fruit morphology, a key trait in *Cucumis* breeding, differs greatly among the species of the Asian/Australian clade (Fig. 3). Early-diverging species, such as the Australian sister clade of *C. javanicus* and several species from India and the Eastern Himalayas (*C. hystrix*, *C. sativus* var. *hardwickii*, *Cucumis debilis*, *C. setosus*, *C. silentvalleyi*, *C. indicus*), have more or less ellipsoid fruits that stay green or turn yellow-orange at maturity. By contrast, the *Cucumis ritcheii*/*C. maderaspatanus* clade is characterized by smooth, round fruits that turn red at maturity.

## Age of Asian/Australian *Cucumis* Clade and Ancestral Area Reconstruction.

A relaxed molecular clock for the Asian/Australian radiation of *Cucumis*, calibrated with a secondary calibration from a Cucurbitaceae clock study that used three fossil and one geological calibration (36), indicates that the lineage comprising melon and cucumber split from its African ancestor  $11.9 \pm 2.0$  Ma ago (Fig. S1). Ancestral area reconstruction under maximum likelihood yields Asia as the area of the most recent common ancestor of melon and cucumber (Fig. S2). The split between melon and its Australian sister species, *C. picrocarpus*, occurred approximately  $2.8 \pm 1.0$  Ma, and that between cucumber and its sister species *C. hystrix*, approximately  $4.6 \pm 1.4$  Ma.

## Discussion

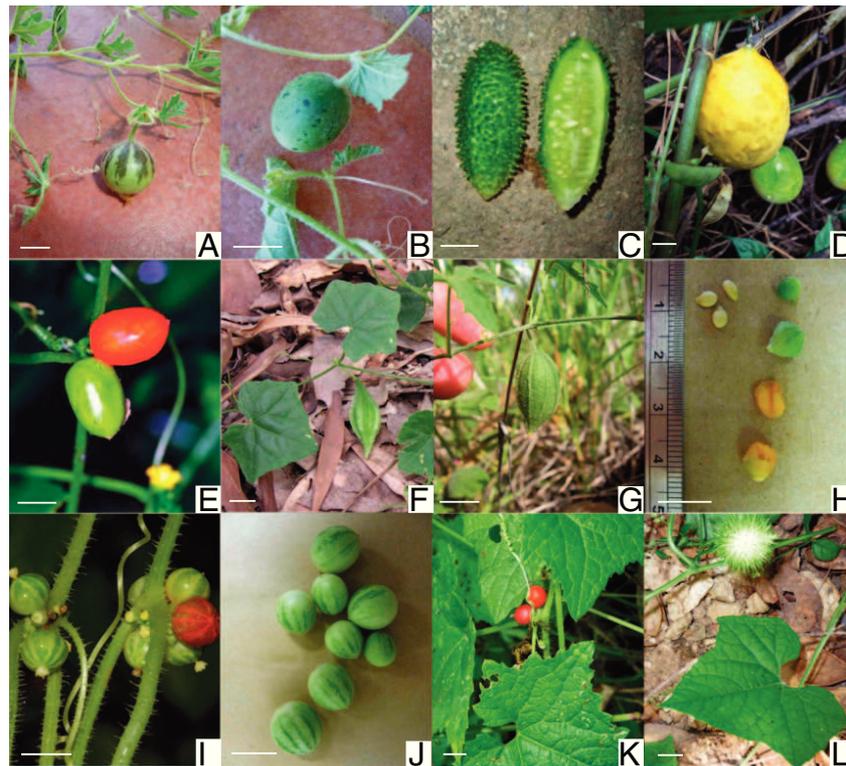
As per this study, *Cucumis* comprises some 25 Asian and Australian species (Fig. 2) in addition to its approximately 30 African species. The increase in Asian/Australian *Cucumis* species, compared with the 12 known in 2008 (32, 33) or the two known in 1993 (17), implies that *Cucumis* was much less understood than hereto



**Fig. 2.** ML tree for 63 taxa of *Cucumis* (Fig. S3 shows a ML tree of all 113 accessions) based on combined sequences from chloroplast and nuclear data (6,202 aligned nucleotides; Table S1), analyzed under a GTR +  $\Gamma$  model. The tree is rooted on *Muellerargia*. Likelihood bootstrap values of at least 75% are given at the nodes; geographic occurrence of species is color-coded (Inset); superscript letters refer to the following: T, species transferred into *Cucumis* (32, 33) based on DNA sequences; S, species never before sequenced; M, specimens formerly identified as *Mukia maderaspatana* (*Cucumis maderaspatanus*); and N, species new to science. Authors of old and new names appear in Table S1.

thought. Few of the Asian and Australian species are in cultivation, most have never had their chromosomes counted, and little is known about their ecology and distribution. A likely reason for

the lack of attention paid to Asian and Australian *Cucumis* is the almost dogmatic view among *Cucumis* specialists that the genus comprised just two Asian species-level taxa (*C. hystrix* and



**Fig. 3.** Habits, fruits, or seeds of (A) *Cucumis picrocarpus*, (B) *C. melo* subsp. *agrestis*, (C) *C. hystrix*, (D) *C. debilis*, (E) *C. javanicus*, (F) *C. costatus*, (G) *C. queenslandicus*, (H) *C. umbellatus*, (I) *C. ritchiei*, (J) *C. villosior*, (K) *C. sp. nov. E*, and (L) *Muellerargia timorensis*. (Scale bar: 1 cm.) Photographs by P. Sebastian (A–C, H, J, K, L) Z. v. Herwijnen (D); B. Wannan (F and G); M. Sardesai (I); and W.J.J.O. de Wilde (reproduced from ref. 35) (E).

*C. sativus*) and that any Australian *Cucumis*-like plants could safely be called *C. melo* (17) or *C. (Mukia) maderaspatanus* (35).

The newly revealed Asian/Australian *Cucumis* radiation of 25 species completely changes the biogeography of the genus. Molecular-clock dating suggests divergence of the Asian/Australian clade from its African relatives at  $11.9 \pm 2.0$  Ma, i.e., during a Miocene period when the African-Arabian plate joined the Asian plate, leading to a closure of the seaway that had previously separated Africa from Asia (37). The ancestor of the Asian/Australian *Cucumis* clade probably spread to Eurasia via this land bridge, as did numerous vertebrates (38). One species, *C. prophetarum*, still ranges from Africa to India and Pakistan, but based on the tree topology (Fig. 2), it dispersed to Asia independently of the ancestor of the *C. sativus/C. melo* clade. The seven species in Australia arose from four dispersal events into that continent, all from Southeast Asia, but at widely different times (Fig. S1).

The DNA phylogeny (Fig. 2) and ancestral area reconstruction (Fig. S2) rejects Kirkbride's (17) grouping of *C. melo* with the African species *C. hirsutus*, *C. humifructus*, and *C. sagittatus* and instead supports the view of 19th century taxonomists (11, 39) that the wild progenitor of melon would be found in India. The Himalayan entities *C. callosus* and *C. trigonus* (names often synonymized with each other and/or with *C. melo*) (17, 40, 41) produce fertile F1 offspring when crossed with *C. melo* (34) and clearly are the wild progenitor from which melon was domesticated. The melon land races occurring in South and East Asia exhibit high genetic diversity (23–26, 42) and deserve to be sampled more densely in future studies. The surprising finding that an Australian species (*C. picrocarpus*) is the sister of the *C. melo/C. callosus/C. trigonus* complex underscores how little is known of the Australian Cucurbitaceae diversity. Most native Australian cucurbits have close relatives among tropical Asian and even Eurasian lineages (e.g., *Austrobryonia*, *Diplocyclos*, *Nealsomitra*, *Neoachmantra*, *Trichosanthes*) and reached Australia from the north (43). It is

possible that other relatives of *C. melo* exist in under-collected regions between India and Australia or may be hiding among unidentified or misclassified material in herbaria.

Most of the approximately 66 species of *Cucumis* now known are monoecious annuals, but dioecious mating systems and a perennial habit evolved several times within the genus. We have begun bringing Asian and Australian species into cultivation to study fruit morphology and chromosomes. The evolution of smooth fruits from spiny fruits (Fig. 3), a traditional key character in *Cucumis*, and the mode of fruit opening are much more plastic than formerly thought (see ref. 28). Overall, the loss of spines appears correlated with a round shape and red color at maturity, probably in connection with bird dispersal. This fits with the inferred dispersal from Asia to Australia, Africa, and various Indian Ocean islands of taxa in the *C. ritchiei/C. maderaspatanus* clade (Fig. 2).

Analyses of the synteny between *C. sativus*, *C. melo* (melon), and *Citrullus lanatus* (watermelon) have revealed that five of the seven chromosomes of *C. sativus* arose by fusions of 10 ancestral chromosomes after the split between *C. sativus* and *C. melo* (14). To more fully understand the rearrangements, it will be useful to now study the chromosomes of other species in the newly revealed Asian/Australian cucumber/melon clade. Study of these species' karyotypes (and other biological traits) will be key in the search for new sources of genes for melon and cucumber improvement. The data on the phylogenetic and geographic relationships of melon and cucumber provided here represent a step toward redirected breeding efforts, which should concentrate on *Cucumis* in Asia and Australia, instead of sub-Saharan Africa. Further population sampling of *C. callosus*, *C. melo*, and *C. trigonus* across Asia will be necessary to assess whether melon was domesticated multiple times.

## Materials and Methods

Fig. 1 shows the locations of 97 of the 113 accessions included in this study (including outgroups). We sequenced five chloroplast markers (the *trnL* intron, the intergenic spacers *trnL-F*, *rpl20-rps12*, and *trnS-G*; and the genes *rbcl* and *matK*) and the nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2, plus the intervening 5.8 S gene using standard procedures (*SI Materials and Methods*). The dataset comprised 6,202 aligned positions. Sequences were edited with Sequencher (version 4.7; Gene Codes) and aligned by eye by using MacClade version 4.0.8 (44). Maximum likelihood (ML) analyses and ML bootstrap searches were performed using RAxML version 7.0.4 (45) (<http://phylobench.vital-it.ch/raxml-bb/>). Tree searches relied on the GTR +  $\Gamma$  model, with model parameters estimated over the duration of specified runs and 100 bootstrap replicates. We carried out Bayesian time estimation with an uncorrelated-rates model, using BEAST version 1.5.3 (46), with a Yule tree prior and the GTR +  $\Gamma$  model with six rate categories. There

are no *Cucumis* fossils, and we therefore used a secondary calibration from a Cucurbitaceae-wide analysis that used four calibration points (36). Mixing of the Markov chain Monte Carlo (MCMC) chain was checked using Tracer version 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) and convergence with AWTY (47). Final trees were edited in FigTree version 1.2.3 (<http://tree.bio.ed.ac.uk/software/figtree/>). Ancestral areas were inferred under maximum likelihood as implemented in Mesquite version 2.6 (<http://mesquiteproject.org/>), using the Markov k-state one-parameter model, which assumes a single rate for all transitions between character states (geographic regions in this case). Further details on methods are provided in *SI Materials and Methods*.

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- Vavilov NI (1935) The phyto-geographical basis for plant breeding. *Origin and Geography of Cultivated Plants* (Cambridge Univ Press, Cambridge), pp 316–366 (English edition); trans Love D (1987).
- Olsen KM, Schaal BA (1999) Evidence on the origin of cassava: Phylogeography of *Manihot esculenta*. *Proc Natl Acad Sci USA* 96:5586–5591.
- Sanjur OJ, Piperno DR, Andres CT, Wessel-Beaver L (2002) Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from a mitochondrial gene: Implications for crop plant evolution and areas of origin. *Proc Natl Acad Sci USA* 99:10923–10928.
- Matsuoka Y, et al. (2002) A single domestication for maize shown by multilocus microsatellite genotyping. *Proc Natl Acad Sci USA* 99:6080–6084.
- Spooner DM, McLean K, Ramsay G, Waugh R, Bryan GJ (2005) A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proc Natl Acad Sci USA* 102:14694–14699.
- Londo JP, Chiang YC, Hung KH, Chiang TY, Schaal BA (2006) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. *Proc Natl Acad Sci USA* 103:9578–9583.
- Fuller DQ (2006) Agricultural origins and frontiers in South Asia: A working synthesis. *J World Prehist* 20:1–86.
- Fuller DQ (2007) Non-human genetics, agricultural origins and historical linguistics in South Asia. *The Evolution and History of Human Populations in South Asia*, eds Petraglia MD, Allchin B (Springer, Berlin), pp 393–443.
- Pokharia AK, Pal JN, Srivastava A (2009) Plant macro-remains from Neolithic Jhusi in Ganga Plain: Evidence for grain-based agriculture. *Curr Sci* 97:546–572.
- Royle JF (1839) *Illustrations of the Botany and other Branches of the Natural History of the Himalayan Mountains* (Wm. H. Allen, London).
- Naudin C (1859) Essais d'une monographie des especes et des variétés du genre *Cucumis*. *Ann Sci Nat Bot* 4:5–87.
- Paris HS, Janick J (2008) What the Roman emperor Tiberius grew in his greenhouses. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, ed Pitrat M (INRA, Avignon, France), pp 33–42.
- FAOSTAT data (2008) *Statistical database (online) of Food and Agriculture Organization of the United Nations*. Available at <http://faostat.fao.org/>. Accessed July 5, 2010.
- Huang S, et al. (2009) The genome of the cucumber, *Cucumis sativus* L. *Nat Genet* 41:1275–1281.
- Benjak A, et al. (2010) A draft sequence of the melon genome revealed by high throughput shotgun sequencing. Plant & Animal Genomes XVIII Conference. Abstracts. Available at [http://www.intl-pag.org/18/abstracts/P01\\_PAGXVIII\\_053.html](http://www.intl-pag.org/18/abstracts/P01_PAGXVIII_053.html).
- Whitaker TW (1933) Cytological and phylogenetic studies in the Cucurbitaceae. *Bot Gaz* 94:780–790.
- Kirkbride JH Jr (1993) *Biosystematic Monograph of the Genus Cucumis (Cucurbitaceae)* (Parkway, Boone, NC).
- Whitaker TW, Davis GN (1962) *Cucurbits: Botany, Cultivation and Utilization* (Interscience, New York).
- Whitaker TW, Bemis WP (1976) Cucurbits, Cucumis, Citrullus, Cucurbita, Lagenaria (Cucurbitaceae). *Evolution of Crop Plants*, ed Simmonds NW (Longman, London), pp 64–69.
- Kerje T, Grum M (2000) The origin of melon, *Cucumis melo*: A review of the literature. *Acta Hort* 510:34–37.
- Périn C, et al. (2002) A reference map of *Cucumis melo* based on two recombinant inbred line populations. *Theor Appl Genet* 104:1017–1034.
- Luan F, Delannay I, Staub JE (2008) Chinese melon (*Cucumis melo* L.) diversity analyses provide strategies for germplasm curation, genetic improvement, and evidentiary support of domestication patterns. *Euphytica* 164:445–461.
- Akashi Y, Fukuda N, Wako T, Masuda M, Kato K (2002) Genetic variation and phylogenetic relationships in East and South Asian melons, *Cucumis melo* L., based on the analysis of five isozymes. *Euphytica* 125:385–396.
- Tanaka K, et al. (2007) Molecular characterization of South and East Asian melon, *Cucumis melo* L., and the origin of group *Conomon* var. *makua* and var. *conomon* revealed by RAPD analysis. *Euphytica* 153:233–247.
- Dhillon NPS, et al. (2007) Diversity among landraces of Indian snapmelon (*Cucumis melo* var. *momordica*). *Genet Resour Crop Evol* 54:1267–1283.
- Dwivedi NK, Dhariwal OP, Krishnan SG, Bhandari DC (2010) Distribution and extent of diversity in *Cucumis* species in the Aravalli ranges of India. *Genet Resour Crop Evol* 57:443–452.
- García-Mas J, Monforte AJ, Arús P (2004) Phylogenetic relationships among *Cucumis* species based on the ribosomal internal transcribed spacer sequence and microsatellite markers. *Plant Syst Evol* 248:191–203.
- Renner SS, Schaefer H, Kocyan A (2007) Phylogenetics of *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evol Biol* 7:58.
- Ghebretinsae AG, Thulin M, Barber JC (2007) Relationships of cucumbers and melons unraveled: Molecular phylogenetics of *Cucumis* and related genera (Benincaseae, Cucurbitaceae). *Am J Bot* 94:1256–1266.
- Renner SS, Schaefer H (2008) Phylogenetics of Cucumis (Cucurbitaceae) as understood in 2008. *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, ed Pitrat M (INRA, Avignon, France), pp 53–58.
- Kocyan A, Zhang LB, Schaefer H, Renner SS (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Mol Phylogenet Evol* 44:553–577.
- Ghebretinsae AG, Thulin M, Barber JC (2007) Nomenclatural changes in *Cucumis* (Cucurbitaceae). *Novon* 17:176–178.
- Schaefer H (2007) *Cucumis* (Cucurbitaceae) must include *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce*: A circumscription based on nuclear and plastid DNA data. *Blumea* 52:165–177.
- Parthasarathy VA, Sambandam CN (1989) Taxonomy of *Cucumis callosus* (Rottl.) Cogn., the wild melon of India. *Cucurbit Genet Coop Rpt* 3:66–67.
- De Wilde WJJO, Duyfjes BEE (2006) *Mukia* Arn. (Cucurbitaceae) in Asia, in particular in Thailand. *Thai Forest Bull (Bot.)* 34:38–52.
- Schaefer H, Heibl C, Renner SS (2009) Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous overseas dispersal events. *Proc Biol Sci* 276:843–851.
- Behrensmeyer AK, Sues H-D, Potts R (1992) *Terrestrial Ecosystems Through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals* (Univ of Chicago Press, Chicago).
- Kappelman J, et al. (2003) Oligocene mammals from Ethiopia and faunal exchange between Afro-Arabia and Eurasia. *Nature* 426:549–552.
- De Candolle A (1883) *Origine des Plantes Cultivées*. (Baillièrre, Paris).
- Chakravarty HL (1959) Monograph on Indian Cucurbitaceae. *Rec Bot Surv India* 17:1–234.
- Jeffrey C (1980) Further notes on Cucurbitaceae V. The Cucurbitaceae of the Indian subcontinent. *Kew Bull* 34:789–809.
- Sujatha VS, Seshadri VS, Srivastava KN, More TA (1991) Isozyme variation in muskmelon (*Cucumis melo* L.). *Indian J Genet Plant Breed* 51:438–444.
- Schaefer H, Telford IRH, Renner SS (2008) *Austrobryonia* (Cucurbitaceae), a new Australian endemic genus, is the closest living relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*. *Syst Bot* 33:125–132.
- Maddison DR, Maddison WP (2003) *MacClade 4.0.8* (Sinauer, Sunderland, MA).
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* 57:758–771.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.
- Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24:581–583.

# Supporting Information

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## SI Materials and Methods

**Plant Materials.** This study samples herbarium material and field-collected plants from most Asian, Australian, and Indian Ocean species described in the genera *Cucumella*, *Cucumis*, *Dicaelospermum*, *Melothria*, or *Mukia*, regardless of whether they were recognized as species in recent monographs or floras. A total of 113 accessions from 61 species (several of them new to science; *Results*) were sequenced (Table S1 provides voucher information and GenBank accession numbers). Fig. 1 shows the geographical distribution of the sampled material. Thirty-four accessions of African *Cucumis* species were included as outgroups (1–3).

**DNA Extraction and Phylogenetic Analyses.** Two hundred seventeen chloroplast sequences (*trnL* intron, *trnL-F*, *rpl20-rps12*, *trnS-trnG* intergenic spacers, *rbcL*, *matK* genes) and 50 nuclear sequences (from the ribosomal DNA internal transcribed spacers ITS1 and ITS2, plus the intervening 5.8 S gene) were newly generated for this study (Table S1). Total genomic DNA was isolated from silica-dried leaves or from herbarium specimens with a commercial plant DNA extraction kit (NucleoSpin; Macherey-Nagel), following the manufacturer's manual. PCR protocols and sequencing followed Kocyan et al. (4). Primers for *rbcL*, *matK*, *rpl20-rps12*, and *trnL* and *trnL-F* were the same as in the study of Kocyan et al. (4). We used the primers of Hamilton (5) for amplification of the *trnS(GCU)-trnG(UCC)* intergenic spacer, and those of Balthazar et al. (6) for the nuclear ITS region. PCR products were purified with a PCR clean-up kit (PCR Wizard; Promega) or using ExoSap (Fermentas) according to the manufacturers' protocols. Cycle sequencing was performed with the BigDye Terminator cycle sequencing kit on an ABI Prism 3100 Avant automated sequencer (Applied Biosystems). Sequencing primers were the same as those used for DNA amplification.

Sequences were edited with Sequencher (version 4.7; Gene Codes) and aligned by eye using MacClade version 4.0.8 (7). The aligned plastid matrix comprised 5,478 nucleotides and the aligned ITS matrix comprised 724 nucleotides. ML analyses and ML bootstrap searches (with 100 replicates) were performed using RAxML-VI-HPC version 7.0.4 (8) (<http://phylobench.vital-it.ch/raxml-bb/>). RAxML searches relied on the GTR +  $\Gamma$  model, with model parameters estimated over the duration of specified

runs. Analysis of the separate plastid and nuclear datasets yielded congruent tree topologies, with differences restricted to nodes with low support (bootstrap <75%), and the datasets were therefore concatenated. To detect if analyses had become trapped in local optima, we conducted 10 separate ML searches and selected the tree with the best likelihood score as the optimal tree.

**Molecular Clock Analyses.** We used Bayesian time estimation with a relaxed clock uncorrelated-rates model, using BEAST version 1.5.3 (9). The dataset used for the dating comprised the chloroplast and nuclear sequences (6,202 aligned nucleotides) of 47 accessions, and analyses used a Yule tree prior and the GTR +  $\Gamma$  model with six rate categories. There are no *Cucumis* fossils, and we therefore used a secondary calibration from a Cucurbitaceae-wide analysis that relied on four calibration points (10). Based on this chronogram (10), we used the following constraints, each with a normal prior distribution and a SD of 3 million years: The *Muellerargia/Cucumis* stem node was set to 16 Ma, the split between the two *Muellerargia* species to 12 Ma, and the split between *C. hirsutus/C. humifructus* and the remaining species of *Cucumis* to 14 Ma (constrained nodes are marked in Fig. S1). MCMC chains were run for 50 million generations, sampling every 1,000th generation. Of the 50,001 posterior trees, we left out the first 12,500 as burn-in. Mixing of the MCMC chain was checked using Tracer version 1.5 (<http://beast.bio.ed.ac.uk/Tracer/>) and convergence with AWTY (11). Final trees were edited in FigTree version 1.2.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

**Ancestral Area Reconstruction.** To estimate the biogeographic history of *Cucumis*, we used ancestral area reconstruction. Ancestral states for the character "geographic region" were inferred under maximum likelihood, using Mesquite version 2.72 (<http://mesquiteproject.org>). We used the Markov k-state one-parameter model, which assumes a single rate for all transitions between character states, and let Mesquite estimate the transition parameters of the model, based on the ML branch lengths and tip trait states in a 63-accession tree with one accession per taxon. The coded geographic regions were: (i) Asia, (ii) Africa/Indian Ocean, (iii) Australia/New Guinea, and (iv) unknown. *C. melo* was coded as unknown.

1. Renner SS, Schaefer H, Kocyan A (2007) Phylogenetics of *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evol Biol* 7:58.
2. Ghebretinsae AG, Thulin M, Barber JC (2007) Relationships of cucumbers and melons unraveled: Molecular phylogenetics of *Cucumis* and related genera (Benincaseae, Cucurbitaceae). *Am J Bot* 94:1256–1266.
3. Renner SS, Schaefer H (2008) Phylogenetics of *Cucumis* (Cucurbitaceae) as understood in 2008. In *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, ed Pitrat M (INRA, Avignon, France), pp 53–58.
4. Kocyan A, Zhang LB, Schaefer H, Renner SS (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Mol Phylogenet Evol* 44:553–577.
5. Hamilton MB (1999) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Mol Ecol* 8:513–525.
6. von Balthazar M, Endress PK, Qiu Y (2000) Phylogenetic relationships in Buxaceae based on nuclear internal transcribed spacers and plastid *ndhF* sequences. *Int J Plant Sci* 161:785–792.
7. Maddison DR, Maddison WP (2003) *MacClade 4.0.8* (Sinauer, Sunderland, MA).
8. Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* 57:758–771.
9. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.
10. Schaefer H, Heibl C, Renner SS (2009) Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous overseas dispersal events. *Proc Biol Sci* 276:843–851.
11. Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24:581–583.





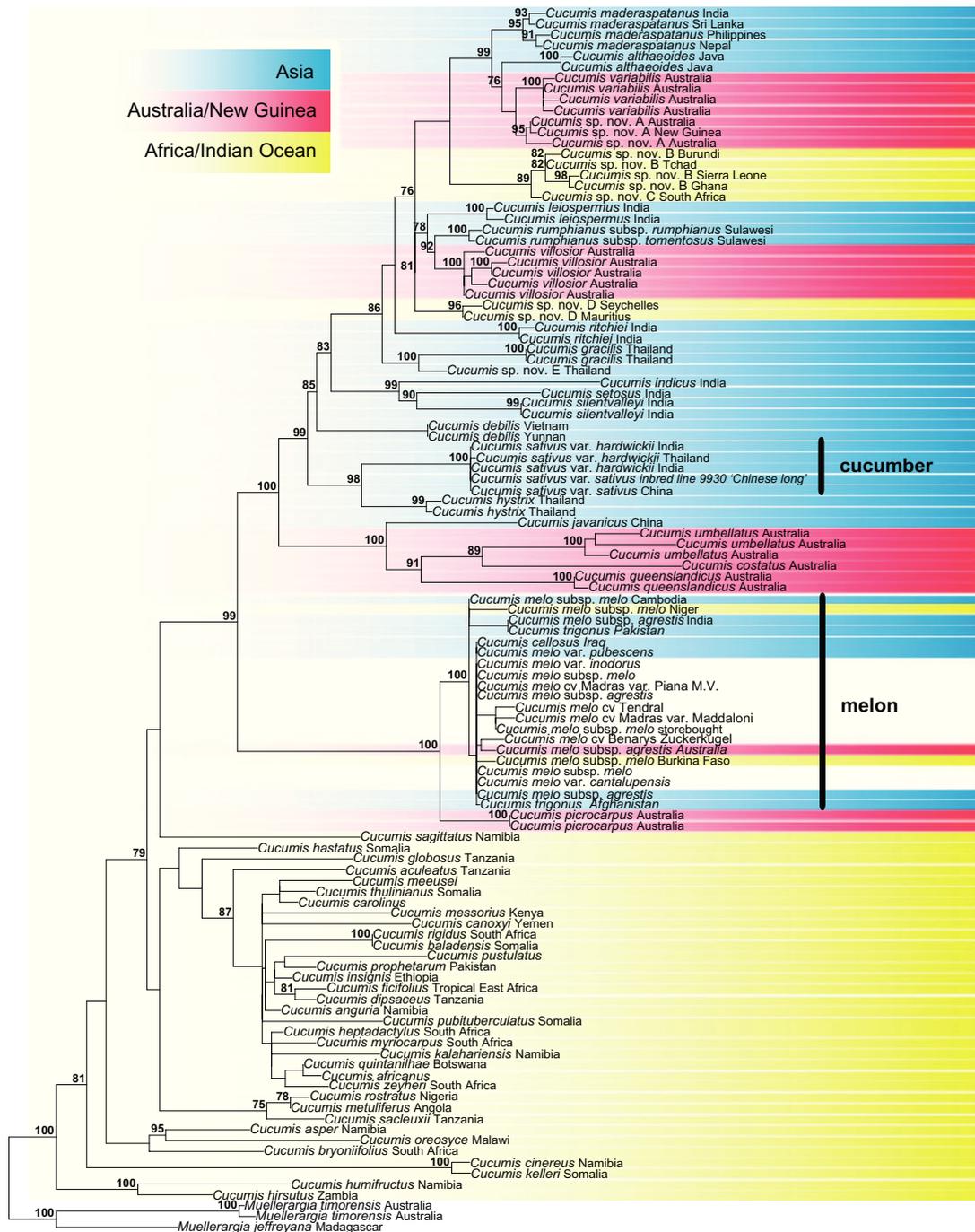


Fig. S3. ML tree for all 113 accessions of *Cucumis*/*Muellerargia* included in this study, based on combined sequences from chloroplast and nuclear data (6,202 aligned nucleotides; Table S1) and analyzed under a GTR +  $\Gamma$  model. The tree is rooted on *Muellerargia* based on Renner and Schaefer (1). Likelihood bootstrap values of at least 75% are given at the nodes. The geographic origin of each accession follows the species name. Authors of old and new names appear in Table S1.

1. Renner SS, Schaefer H (2008) Phylogenetics of *Cucumis* (Cucurbitaceae) as understood in 2008. In *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, ed Pitrat M (INRA, Avignon, France), pp 53–58.

## Other Supporting Information

[Table S1 \(DOC\)](#)

**Table S1:** Species, sources and geographic origin of the sequenced material, nuclear and chloroplast loci used, and GenBank accession numbers of sequences included into this study. Asterisks (\*) mark sequences created newly for this study. Herbarium acronyms follow the Index Herbariorum at <http://sciweb.nybg.org/science2/>

Species	DNA source	Geogr. origin of sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>rbcL</i> gene	<i>matK</i> gene
<i>Cucumis aculeatus</i> Cogn.	Kuchar 22468 (MO)	Tanzania	EF595865						
<i>Cucumis africanus</i> L.f.	AGG 1009 (MO) USDA, ARS Reg. Plant Introd. Station; Ames, Iowa, USA	unknown	EF595866						
<i>Cucumis althaeoides</i> (Ser.) I. Telford & P. Sebastian comb. nov. ined.	A.R. Insani SAN10 (L)	Java	HM596903*	HM597010*	HM597010*	HM596960*	HM597063*		
<i>Cucumis althaeoides</i> (Ser.) I. Telford & P. Sebastian comb. nov. ined.	H. Raap 499 (L)	Java	HM596904*	HM597011*	HM597011*	HM596961*	HM597064*		
<i>Cucumis anguria</i> L.	R. Seydel 3439 (M)	Namibia		DQ785869	DQ785869	DQ785855	HM597065*	DQ785827	DQ785843
<i>Cucumis asper</i> Cogn.	O.H. Volk 2789 (M)	Namibia	EF091850	DQ785868	DQ785868	DQ785854		DQ785826	DQ785842
<i>Cucumis baladensis</i> Thulin	M. Thulin <i>et al.</i> 7464 (UPS)	Somalia	EF595869						
<i>Cucumis bryoniifolius</i> (Merxm.) Ghebret. & Thulin	M. Wilkins 214b (seeds cult. in Tucson, Arizona)	South Africa	EF091851	DQ536763	DQ536763	DQ648165	HM597066*	DQ535798	DQ536657
<i>Cucumis callosus</i> Cogn.	PI435284, NCRPIS, Ames, Iowa, USA	Iraq	AJ488211						
<i>Cucumis canoxyi</i> Thulin & A.N. Al-Gifri	M. Thulin <i>et al.</i> 9864 (UPS)	Yemen	EF595870						
<i>Cucumis carolinus</i> J.H. Kirkbr.	AGG 1017 (MO) Inst. de la Rech. Agronom., Avignon	unknown	EF595871						
<i>Cucumis cinereus</i> (Cogn.) Ghebret. & Thulin	Maggis <i>et al.</i> 1026 (WIND)	Namibia	EF595863						

<i>Cucumis costatus</i> I. Telford sp. nov. ined.	P.I. Forster 9514 (NE) = HS414 (Renner <i>et al.</i> , 2007)	Australia: QLD	EF174483	EF174486	EF174486	EF174482	HM597067*	EF174480	EF174478
<i>Cucumis debilis</i> W.J. de Wilde & Duyfjes	A. Pételot 2193 (P)	Vietnam: Sa Pa					HM597012*		
<i>Cucumis debilis</i> W.J. de Wilde & Duyfjes	T. Tu 514 (M)	China: Yunnan	HM596905*	HM597013*	HM597013*	HM596962*	HM597068*		
<i>Cucumis dipsaceus</i> Ehrenb. ex Spach	H. Schaefer 05/510 (M)	Tanzania	EF093513	DQ785870	DQ785870	DQ785856	HM597069*	DQ785828	DQ785844
<i>Cucumis ficifolius</i> A. Rich.	J.E. Weiss s.n. (M)	Tropical East Africa		DQ785871	DQ785871	DQ785857	HM597070*	DQ785829	DQ785845
<i>Cucumis globosus</i> C. Jeffrey	Kayombo 681 (MO)	Tanzania	EF595874						
<i>Cucumis gracilis</i> (Kurz) Ghebret. & Thulin	C.F. van Beusekom <i>et al.</i> 3567 (MO)	Thailand: Kachanaburi	HM596907*		HM597015*	HM596964*	HM597072*		
<i>Cucumis gracilis</i> (Kurz) Ghebret. & Thulin	P. Phonsena <i>et al.</i> 5651 (L)	Thailand: Mae Hong Son	HM596906*	HM597014*	HM597014*	HM596963*	HM597071*		
<i>Cucumis hastatus</i> Thulin	Kuchar 17327 (K)	Somalia	EF595875						
<i>Cucumis heptadactylus</i> Naudin	W. Giess 168 (M)	South Africa		DQ785872	DQ785872	DQ785858	HM597073*	DQ785830	DQ785840
<i>Cucumis hirsutus</i> Sond. in Harv. & Sond.	N.B. Zimba 874 (MO)	Zambia		DQ536804	DQ536804	DQ536542	HM597074*	DQ535799	DQ536658
<i>Cucumis humifructus</i> Stent	H. Merxmueller, W. Giess 30150	Namibia	EF093514	DQ785873	DQ785873	DQ785873	HM597075*	DQ785831	DQ785841
<i>Cucumis hystrix</i> Chakrav.	P. Sebastian 9 (M)	Thailand: Mae Sai	HM596908*	HM597016*	HM597016*	HM596965*	HM597076*		
<i>Cucumis hystrix</i> Chakrav.	S. Suddee <i>et al.</i> 2503 (L)	Thailand	EF093515	HM597017*	DQ785874	DQ785860	HM597077*	DQ785832	DQ785846
<i>Cucumis indicus</i> Ghebret. & Thulin	D. Ritchie 67 (E)	India: Eastern Ram Ghat	HM596909*	HM597018*	HM597018*	HM596966*	HM597078*		
<i>Cucumis insignis</i> C. Jeffrey	Gilbert <i>et al.</i> 8061 (UPS)	Ethiopia	EF595880						
<i>Cucumis javanicus</i> (Miq) Ghebret. & Thulin	H. Schaefer 05/133 (M)	China: Yunnan	EF174484	EF174485	EF174485	EF174481	HM597079*	EF174479	EF174477
<i>Cucumis kalahariensis</i> A. Meeuse	Maggis 1036 (WIND)	Namibia	EF595881						
<i>Cucumis kelleri</i> (Cogn.) Ghebret. & Thulin	M. Thulin <i>et al.</i> 10578 (UPS)	Somalia	EF595864						

Species	DNA source	Geogr. origin of sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>rbcL</i> gene	<i>matK</i> gene
<i>Cucumis leiospermus</i> (Wight & Arn.) Ghebret. & Thulin	K.M. Matthew <i>et al.</i> 46998 (AAU)	India: Tamil Nadu	HM596910*	HM597019*	HM597019*	HM596967*	HM597080*		
<i>Cucumis leiospermus</i> (Wight & Arn.) Ghebret. & Thulin	R. Wight 1112b (E)	India: Kerala	HM596911*		HM597020*	HM596968*	HM597081*		
<i>Cucumis maderaspatanus</i> L.	S. Siddarthan s.n. (M)	India: Tamil Nadu	HM596915*	HM597024*	HM597024*	HM596972*	HM597085*		
<i>Cucumis maderaspatanus</i> L.	J.H. de Haas 2011 (L)	Nepal: Kathmandu	HM596912*		HM597021*	HM596969*	HM597082*		
<i>Cucumis maderaspatanus</i> L.	M. Ramos & G. Edano 30939 (K)	Philippines: Panay Island	HM596914*	HM597023*	HM597023*	HM596971*	HM597084*		
<i>Cucumis maderaspatanus</i> L.	D. Philcox <i>et al.</i> 10540 (K)	Sri Lanka	HM596913*	HM597022*	HM597022*	HM596970*	HM597083*	HM596953*	
<i>Cucumis meeusei</i> C. Jeffrey	AGG 1022 (MO) USDA Torrey Pines Station, Lakolla, California		EF595882						
<i>Cucumis melo</i> L. subsp. <i>agrestis</i> (Naudin) Pangalo	A. Ali & A. Pandey 1019 (BHAG)	India	GQ183039						
<i>Cucumis melo</i> L. subsp. <i>agrestis</i> (Naudin) Pangalo	I.R. Telford 11472 (CBG)	Australia: QLD	HM596916*	HM597025*	HM597025*	HM596973*	HM597086*		
<i>Cucumis melo</i> L. subsp. <i>agrestis</i> (Naudin) Pangalo	AGG 1028 (MO) USDA ARS Reg. Plant Introd. Stat, Ames, Iowa, USA	unknown	EF595883						
<i>Cucumis melo</i> L. subsp. <i>agrestis</i> (Naudin) Pangalo	PI 161375 NCRPIS, Ames, Iowa, USA	unknown	AJ488227						
<i>Cucumis melo</i> L. subsp. <i>melo</i>	store-bought cantaloupe	unknown		DQ536764	DQ536764	DQ648166	HM597087*	DQ535800	DQ536659
<i>Cucumis melo</i> L. subsp. <i>melo</i>	H. Schaefer 2010/20 (K)	Cambodia	HM596917*						
<i>Cucumis melo</i> L. subsp. <i>melo</i>	E.G. Achigan-Dako 71ku77 (GAT)	Niger	AM981118						
<i>Cucumis melo</i> L. subsp. <i>melo</i>	T111 Semillas Fitó S.A.	unknown	AJ488233						

<i>Cucumis melo</i> L. subsp. <i>melo</i>	E.G. Achigan-Dako 25ae046 (GAT)	Burkina Faso	AM981117						
<i>Cucumis melo</i> L. subsp. <i>melo</i>	AGG 1031 (MO) USDA ARS Reg. Plant Introd. Stat, Ames, Iowa, USA	unknown	EF595886						
<i>Cucumis melo</i> L. var. <i>pubescens</i> (Willd.) Kurz	CUC48/1991 Institute of Plant Genetics and Crop Plant Research (Germany)	unknown	AJ489229						
<i>Cucumis melo</i> L. var. <i>indorus</i> H. Jacq	AGG 1030 (MO) USDA ARS Reg. Plant Introd. Stat, Ames, Iowa, USA	unknown	EF595885						
<i>Cucumis melo</i> L. var. <i>cantaloupensis</i> Naudin	AGG 1029 (MO) USDA ARS Reg. Plant Introd. Stat, Ames, Iowa, USA	unknown	EF595884						
<i>Cucumis melo</i> L. cv Madras var. Maddaloni		unknown	EU312158						
<i>Cucumis melo</i> L. cv Madras var. Piana M.V.		unknown	EU312159						
<i>Cucumis melo</i> L. cv Tendral		unknown	EF312157						
<i>Cucumis melo</i> L. cv. Benarys Zuckerkugel	store-bought cantaloupe	unknown	AF01333						
<i>Cucumis messorius</i> (C. Jeffrey) Ghebret.& Thulin	P.R.O. Bally B15187 (EA)	Kenya	EF093527	DQ535872		DQ536572			DQ536706
<i>Cucumis metuliferus</i> E. Mey. ex Naudin	B. de Winter, W. Marais 4614 (M)	Angola	EF093517	DQ785876	DQ785876	DQ785862	HM597088*	DQ785834	DQ785848
<i>Cucumis myriocarpus</i> Naudin	S.S. Renner 2801 (M)	South Africa	EF093518	DQ785878	DQ785878	DQ785864	HM597089*	DQ785836	DQ785850
<i>Cucumis oreosyce</i> H. Schaeff.	E. Phillips 2821 (Z)	Malawi		DQ536845	DQ536845	DQ536576	HM597090*	DQ535833	DQ536711
<i>Cucumis picrocarpus</i> F. Muell	I.R. Telford (CBG)	Australia: NT	HM596919*	HM597027*	HM597027*	HM596975*	HM597092*		
<i>Cucumis picrocarpus</i> F. Muell	A.A. Mitchell 3076 (CBG)	Australia: NT	HM596918*	HM597026*	HM597026*	HM596974*	HM597091*		

Species	DNA source	Geogr. origin of sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>rbcL</i> gene	<i>matK</i> gene
<i>Cucumis prophetarum</i> L. subsp. <i>prophetarum</i>	K.H. Rechinger 28768 (M)	Pakistan	EF093519	DQ785879	DQ785879	DQ785865	HM597093*	DQ785837	DQ785851
<i>Cucumis pubituberculatus</i> Thulin	M. Thulin 6321 (UPS)	Somalia	EF595890						
<i>Cucumis pustulatus</i> Naudin ex Hook.f.	AGG 1023 (MO) USDA ARS Reg. Plant Introd. Stat, Ames, Iowa, USA	unknown	EF595891						
<i>Cucumis queenslandicus</i> I. Telford sp. nov. ined.	I.R. Telford, P. Sebastian 13316 (M)	Australia: QLD	HM596921*	HM597029*	HM597029*	HM596977*	HM597094*		
<i>Cucumis queenslandicus</i> I. Telford sp. nov. ined.	P.I. Forster 12812 (BRI)	Australia: QLD	HM596920*	HM597028*	HM597028*	HM596976*			
<i>Cucumis quintanilhae</i> R.Fern. & A.Fern.	AGG 019 (UAE)	Botswana	EF595892						
<i>Cucumis rigidus</i> E. Mex ex Sond. In Harv. & Sond.	Pearson 4023 (MO)	South Africa	EF595893						
<i>Cucumis ritchiei</i> (C.B.Clarke) Ghebret. & Thulin	H. Santapaa 13354 (MO)	India: Khandala	EF093524	DQ536811	DQ536811	DQ536546	HM597096*	DQ535806	
<i>Cucumis ritchiei</i> (C.B.Clarke) Gheret. & Thulin	A. Meebold 9452 (WRSL)	India	HM596922*	HM597030*	HM597030*	HM596978*	HM597095*		
<i>Cucumis rostratus</i> J.H. Kirkbr.	Babuker 8712 (MO)	Nigeria	EF595894						
<i>Cucumis rumphianus</i> (Schaeff) H. Schaeff subsp. <i>tomentosus</i> de Wilde & Duyfjes	W.J.J.O. de Wilde & B.E.E Duyfjes 21757 (L)	Sulawesi: Rantapao	HM596924*	HM597032*	HM597032*	HM596980*	HM597098*		
<i>Cucumis rumphianus</i> (Scheff.) H. Schaeff subsp. <i>rumphianus</i>	J. J. Wieringa 1872 (K)	Sulawesi	HM596923*		HM597031*	HM596979*	HM597097*		
<i>Cucumis saclexii</i> Pailleux & Bois	H. Schaefer 05/411 (M)	Tanzania	EF093520	DQ785880	DQ785880	DQ785866	HM597099*	DQ785838	DQ785852
<i>Cucumis sagittatus</i> Peyr. in Wawra & Peyr.	D. Decker-Walters 1124 (FTG)	Namibia	EF093521	DQ536806	DQ536806	DQ648168	HM597100*	DQ535802	DQ536661

<i>Cucumis sativus</i> L.	'Chinese Long' Inbred Line 9930 (Huang <i>et al.</i> 2009)	unknown	HM596928*	HM597036*	HM597036*	HM596984*	HM597104*		
<i>Cucumis sativus</i> L.	S. Renner 2745 <sup>1</sup> (M); 2822 <sup>2</sup> cult. BG Munich	unknown <sup>1</sup> /China <sup>2</sup>	EF093522 <sub>2</sub>	DQ53676 <sub>1</sub>	DQ536765 <sub>1</sub>	DQ648169 <sub>1</sub>	HM597105* <sub>1</sub>	DQ53574 <sub>1</sub>	DQ53666 <sub>1</sub>
<i>Cucumis sativus</i> L. var. <i>hardwickii</i> (Royle) Gabaev	P. Phonsena, B.E.E. Duyfjes, W.J.J.O. deWilde, Chusithong 5654 (L)	Thailand: Mae Hong Son	HM596925*	HM597033*	HM597033*	HM596981*	HM597101*		
<i>Cucumis sativus</i> L. var. <i>hardwickii</i> (Royle) Gabaev	PI 214155, RijkZwaan Breeding BV Delft, NL	India: Karnataka	HM596926*	HM597034*	HM597034*	HM596982*	HM597102*		
<i>Cucumis sativus</i> L. var. <i>hardwickii</i> (Royle) Gabaev	PI 215589; RijkZwaan Breeding BV. Delft, NL	India: Uttar Pradesh	HM596927*	HM597035*	HM597035*	HM596983*	HM597103*		
<i>Cucumis setosus</i> Cogn.	D. Ritchie 321 (E)	India	HM596929*		HM597037*	HM596985*	HM597106*		
<i>Cucumis silentvalleyi</i> (Manilal, T. Sabu & P. Mathew) Ghebret. & Thulin	K.S. Manilal 10662 (K)	India: Kerala	HM596930*	HM597038*	HM597038*	HM596986*			
<i>Cucumis silentvalleyi</i> (Manilal, T. Sabu & P. Mathew) Ghebret. & Thulin	J. Sinclair 3589 (E)	India: Kerala	HM596931*	HM597039*	HM597039*	HM596987*			
<i>Cucumis</i> sp. nov. A	K. Brennan 2576 (DNA)	Australia: NT	HM596932*	HM597040*	HM597040*	HM596988*	HM597107*	HM596954*	
<i>Cucumis</i> sp. nov. A	H.S. McKee 8320 (L)	Australia: NT	HM596933*	HM597041*	HM597041*	HM596989*	HM597108*		
<i>Cucumis</i> sp. nov. A	R. Pullen 6804 (L)	Papua New Guinea: Little Mount Lawes	HM596934*		HM597042*	HM596990*	HM597109*		
<i>Cucumis</i> sp. nov. B	W.F. Rodenburg 58 (L)	Ghana: Kpandu	HM596936*	HM597045*	HM597045*	HM596993*	HM597111*	HM596955*	
<i>Cucumis</i> sp. nov. B	H. Schaefer 06/434 (M)	Sierra Leone	HM596937*	HM597046*	HM597046*	HM596994*	HM597112*		
<i>Cucumis</i> sp. nov. B	J. Lewalle 2997 (M)	Burundi	HM596935*	HM597044*	HM597044*	HM596992*	HM597110*		

Species	DNA source	Geogr. origin of sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>rbcL</i> gene	<i>matK</i> gene
<i>Cucumis</i> sp. nov. B	J. Léonard 3443 (M)	Chad		HM597043*	HM597043*	HM596991*			
<i>Cucumis</i> sp. nov. C	R.G. Strey 6468 (M)	South Africa: Natal	HM596938*	HM597047*	HM597047*	HM596995*	HM597113*		
<i>Cucumis</i> sp. nov. D	J. Gueho & F. Staub 11542 (K)	Mauritius: St. Brandon	HM596939*		HM597048*	HM596996*	HM597114*		
<i>Cucumis</i> sp. nov. D	D.R. Stoddart & M.E.D. Poore 1340 (K)	Seychelles: Farquhar Atoll, North Island			HM597049*	HM596997*	HM597115*		
<i>Cucumis</i> sp. nov. E	J.F. Maxwell 02/434 (CMU)	Thailand: Chiang Mai	EF093526	DQ536843	DQ536843	DQ648182		DQ535761	DQ536705
<i>Cucumis thulinianus</i> J.H. Kirkbr.	Yohannes 3611 (UAE)	Somalia	EF595900						
<i>Cucumis trigonus</i> Roxb.	Ames 24297 NCRPIS, Ames, Iowa, USA	Pakistan	AJ488234						
<i>Cucumis trigonus</i> Roxb.	D. Podlech 32603 (M)	Afghanistan	EF093516	DQ785875	DQ785875	DQ785861	HM597116*	DQ785833	DQ785847
<i>Cucumis umbellatus</i> I. Telford sp. nov. ined.	L.A. Craven & G.M. Wightman 8363 (CANB)	Australia: NT	HM596940*		HM597050*	HM596998*	HM597117*		
<i>Cucumis umbellatus</i> I. Telford sp. nov. ined.	P. Sebastian 15 (M)	Australia: NT	HM596942*	HM597052*	HM597052*	HM597000*	HM597119*		
<i>Cucumis umbellatus</i> I. Telford sp. nov. ined.	Sands 4576 (K)	Australia: WA	HM596941*	HM597051*	HM597051*	HM596999*	HM597118*		
<i>Cucumis variabilis</i> I. Telford & P. Sebastian sp. nov. ined.	K.M. Allan 469 (CBG)	Australia: WA	HM596943*		HM597053*	HM597001*	HM597120*		
<i>Cucumis variabilis</i> I. Telford & P. Sebastian sp. nov. ined.	K. Newbey 10312 (K)	Australia: WA	HM596944*	HM597054*	HM597054*		HM597121*		
<i>Cucumis variabilis</i> I. Telford & P. Sebastian sp. nov. ined.	I.R. Telford, G. Butler 6476 (CBG)	Australia: WA	HM596945*	HM597055*	HM597055*	HM597002*	HM597122*		
<i>Cucumis variabilis</i> I. Telford & P. Sebastian sp. nov. ined.	P.G. Wilson 8389 (L)	Australia: WA	HM596946*	HM597056*	HM597056*	HM597003*	HM597123*		

<i>Cucumis villosior</i> (Cogn.) I. Telford & P. Sebastian comb. et stat. nov. ined.	M.B. Thomas, W. Johnson s.n. (DNA)	Australia: QLD	HM596950*	HM597060*	HM597060*	HM597007*	HM597127*	HM596957*	
<i>Cucumis villosior</i> (Cogn.) I. Telford & P. Sebastian comb. et stat. nov. ined.	B.G. Thomson 218 (DNA)	Australia: NT	HM596951*		HM597061*	HM597008*	HM597128*	HM596958*	
<i>Cucumis villosior</i> (Cogn.) I. Telford & P. Sebastian comb. et stat. nov. ined.	T.S. Henshall 3450 (DNA)	Australia: NT	HM596948*	HM597058*	HM597058*	HM597005*	HM597125*		
<i>Cucumis villosior</i> (Cogn.) I. Telford & P. Sebastian comb. et stat. nov. ined.	B. Barnsley 1656 (CBG)	Australia: QLD	HM596947*	HM597057*	HM597057*	HM597004*	HM597124*	HM596956*	
<i>Cucumis villosior</i> (Cogn.) I. Telford & P. Sebastian comb. et stat. nov. ined.	Telford I.R., Butler G. 6028 (CBG)	Australia: WA	HM596949*	HM597059*	HM597059*	HM597006*	HM597126*		
<i>Cucumis zeyheri</i> Sond. in Harv. & Sond.	D. Decker-Walters 1114 (FTG)	South Africa	EF093523	DQ536807	DQ536807	DQ648170	HM597129*	DQ535803	DQ536663
<i>Muellerargia jeffreyana</i> Keraudren	G. Cours 5586 (P)	Madagascar		EU436361	EU436361	EU436337		EU436387	EU436411
<i>Muellerargia timorensis</i> Cogn.	I.R. Telford, P. Sebastian 13307 (M)	Australia: QLD	HM596952*	HM597062*	HM597062*	HM597009*	HM597130*	HM596959*	
<i>Muellerargia timorensis</i> Cogn.	D.L. Jones 3666 (NE)	Australia: QLD	EF093525	DQ536842	DQ536842	DQ536571		DQ535777	DQ536704

Huang S, *et al.* (2009) The genome of cucumber, *Cucumis sativus* L. *Nature Genetics*, 41:1275–1281.

Renner SS, Schaefer H, Kocyan A (2007) Phylogenetics of *Cucumis* (Cucurbitaceae): cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evolutionary Biology*, 7: 58.



## CHAPTER 2

*CUCUMIS* (CUCURBITACEAE) IN AUSTRALIA AND EASTERN  
MALESIA, INCLUDING NEWLY RECOGNIZED SPECIES AND THE  
SISTER SPECIES TO *C. MELO*

TELFORD, I.R.H., SEBASTIAN, P., BRUHL, J.J. AND RENNER, S.S. 2011.  
*Systematic Botany*, 36: 376-389.



## *Cucumis* (Cucurbitaceae) in Australia and Eastern Malesia, Including Newly Recognized Species and the Sister Species to *C. melo*

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**Abstract**—Molecular phylogenetic analyses based on numerous plant accessions have shown that *Cucumis* comprises 25 species in Asia, the Malesian region, and Australia, rather than just two as traditionally thought. Among the 25 species several are new, and here we describe four from tropical Australia. The new species *C. costatus*, *C. queenslandicus*, *C. umbellatus*, and *C. variabilis* are illustrated, their distributions are mapped based on 7–50 collections per species, and information is provided on habitats and conservation status. We also validate *C. althaeoides* comb. nov. and *C. argenteus* comb. nov., update the description of *C. picrocarpus*, and provide a key to the 11 native and naturalized species of *Cucumis* occurring in Australia and eastern Malesia. The Australasian species diversity of *Cucumis*, a genus that until recently was held to be essentially African, is of interest also because Australia harbours the sister species to the commercially important melon, *C. melo*.

**Keywords**—*Cucumis picrocarpus*, melon, morphological characters, phylogenetics, sequencing herbarium specimens, taxonomy.

Phylogenetic reconstructions for the Cucurbitaceae have revealed unexpected relationships for Australian species and genera (Kocyan et al. 2007). Of particular interest is the discovery that *Cucumis* as traditionally conceived (Kirkbride 1993) is paraphyletic and that it comprises 25, rather than two, Asian, Malesian, and Australian species (Renner et al. 2007; Renner and Schaefer 2008; Sebastian et al. 2010). A particularly poorly understood African/Asian/Australian genus found embedded in *Cucumis* is *Mukia* Arn. In the last comprehensive treatment of the Australian Cucurbitaceae (Telford 1982), *Mukia* was presented as containing six species in Australia: the putatively widespread and polymorphic *M. maderaspatana* (L.) M. Roem. (now *Cucumis maderaspatanus* L.), *M. micrantha* (F. Muell.) F. Muell. (now *Austrobryonia micrantha* (F. Muell.) I. Telford), and five species not validly published but assigned code names. Based on molecular data, three of these unnamed species, together with *M. micrantha*, form a distinct clade, described as *Austrobryonia* H. Schaefer, a genus of the tribe Bryonieae, an early branch in the Cucurbitaceae (Schaefer et al. 2008). Several other new combinations required by the molecular findings, but not concerning Australian species, have already been made (Ghebretinsae et al. 2007; Schaefer 2007).

Molecular data obtained since 2007 for an increasingly comprehensive sampling of Asian and Australian specimens (over 100 accessions, mostly from herbarium collections) have revealed that the *Cucumis* species treated as *Mukia* sp. A and *M. sp. B* in the *Flora of Australia* (Telford 1982), and a third more recently discovered species, are close relatives, in line with their morphological similarity. These three species are sister to *C. javanicus* (Miq.) Ghebret. & Thulin (Fig. 1). Molecular data generated for the present study also show that *Melothria argentea* is a genetically distinct species of *Cucumis* (Fig. 1). Likewise, a species originally described as *Bryonia althaeoides* by Seringe (1828) and later regarded as a species of *Mukia* (Roemer, 1846) or *Melothria* (Nakai, 1938) turns out to be the sister species to *C. variabilis*, another new Australian species (Fig. 1). Lastly, the native Australian species *C. picrocarpus*, which had long been regarded as a synonym of *C. melo* (Kirkbride 1993), instead is the sister species to melon, *C. melo* (Fig. 1). The geographic origin of *C. melo*

itself and its likely domestication in the Eastern Himalayas region are dealt with in a related paper (Sebastian et al. 2010). Because of the great interest of melon breeders in *C. picrocarpus*, we here provide an updated description of this species based on new observations in the herbarium and field. Lastly, we provide a key to the 11 native and naturalized species of *Cucumis* now known from Australia and adjacent eastern Malesia.

### MATERIALS AND METHODS

**Herbarium Work, Field Observations, Morphological Data**—Specimens in BRI, CANB, DNA, MEL, NE, PERTH and QRS (now in CNS) were examined, and we also obtained scans of types from B, E, G-DC, and PR. Floral measurements were taken on rehydrated dried specimens. Fieldwork by the first two authors greatly improved understanding of the ecological niches of the native Australian species of *Cucumis*.

**Molecular Phylogenetics**—The DNA sequence matrix for this paper is modified from the one used in Sebastian et al. (2010). That study included 113 accessions of *Cucumis* and its sister group, *Muellerargia* Cogn., sequenced for six chloroplast regions (the *trnL* intron, the intergenic spacers *trnL-F*, *rpl20-rps12*, and *trnS-G*; and the genes *rbcl* and *matK*) plus the nrDNA ITS1 and ITS2, and the intervening 5.8 S gene segment. The dataset comprised 6,202 aligned positions, and for the present study, we added sequences for two accessions from Timor (*C. althaeoides* Zippel 107 (L): *rpl20-rps12* intergenic spacer (HQ439182); *Cucumis* sp. nov. collector ignotus 1305 (L): *rpl20-rps12* and *trnS-trnG* intergenic spacers (HQ439180, HQ439181) to resolve the application of the name *C. althaeoides*. In the phylogeny shown here (Fig. 1) we excluded most African *Cucumis* and several undescribed species from mainland Asia and from Africa. The phylogeny in Sebastian et al. (2010: Fig. 2) and the one included here (Fig. 1) are not identical in their taxon sampling. Sequencing approaches and phylogenetic methods are as described in Sebastian et al. (2010). Maximum likelihood (ML) analyses (Felsenstein 1973) and ML bootstrap searches (Felsenstein 1985) were performed using RAxML (Stamatakis et al. 2008).

Voucher information and GenBank accession numbers are listed in Table S1 of Sebastian et al. (2010). The alignment used for the present study has been deposited in TreeBASE (study number S10944).

### RESULTS AND DISCUSSION

Based on the combined nuclear and plastid sequence data and geographic provenience of the samples (Fig. 1), there are

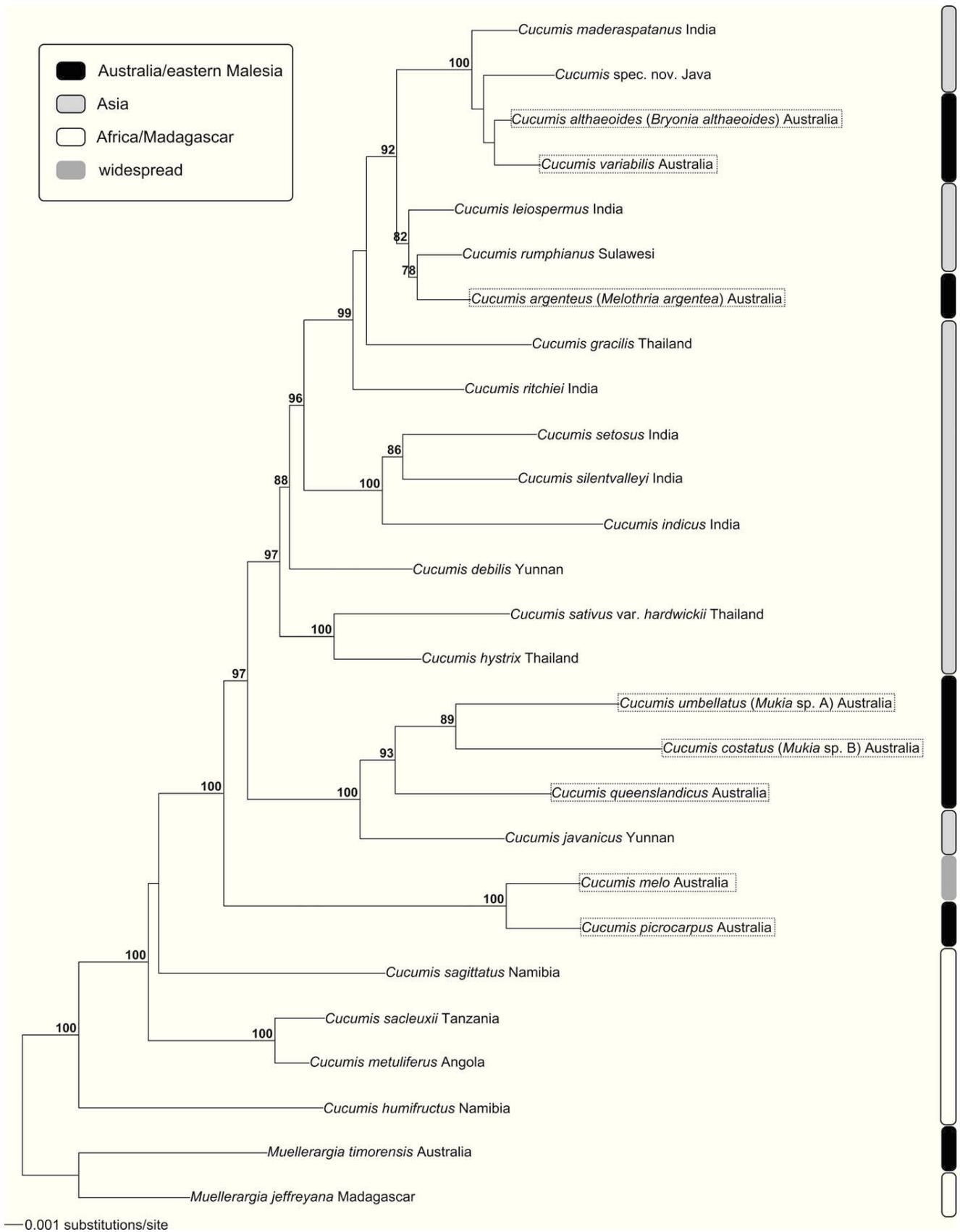


FIG. 1. Maximum likelihood tree for 27 species of *Cucumis* based on combined chloroplast and nuclear sequences; numbers at nodes are bootstrap values  $\geq 75\%$ . The tree is rooted on *Muellerargia*, the sister group of *Cucumis*. Geographic occurrence of species is color-coded (inset), and the geographic origin of each accession follows the species name. Species discussed in the text are marked by boxes.

eight indigenous species of *Cucumis* in Australia, of which one also occurs in Timor and southern New Guinea. *Cucumis maderaspatanus* does not occur in Australia (Sebastian et al. 2010; our Fig. 1), and previous citations of that species for Australia were based on misidentifications. Resolving the circumscription of all biological species hiding under the name *C. maderaspatanus*, however, requires an analysis of specimens from throughout the range of this putatively Asian/African species (De Wilde and Duyfjes 2006). In the present study, we begin this task by comparing relevant Australian specimens and names (Telford 1982), excluding those already transferred to *Austrobryonia* H. Schaefer (Schaefer et al. 2008).

Besides the eight native species of *Cucumis*, three African species have become widely naturalized in Australia, viz. *C. myriocarpus* Naudin, *C. zeyheri* Sond. in Harv. & Sond., and *C. metuliferus* E. Mey. ex Naudin, and there are also sporadically naturalized populations of cultivated melon, *C. melo*, a species that likely evolved in the eastern Himalayan region (Sebastian et al. 2010; contra Ghebretinsae et al. 2007b). Because of the potential interest for *Cucumis* breeders, we include a key to the 11 native and naturalized Australian species, following the taxonomic treatment of the four new species, two new combinations, and an emended description of *C. picrocarpus*.

Species of *Cucumis* native to Australia are descended from five ancestral lineages that arrived from southeast Asia several million years ago (Sebastian et al. 2010 for molecular clock estimates of divergence times in *Cucumis*). One of the five entries into Australia gave rise to *C. costatus*, *C. queenslandicus*, and *C. umbellatus*, while *C. picrocarpus*, *C. melo*, and *C. argenteus* represent independent arrivals to the continent (Fig. 1). The genetically distinct Australian endemic *C. picrocarpus* diverged from *C. melo* about 3 Ma ago (Sebastian et al.

2010), while Australian populations of *C. melo* all represent naturalized cultivated forms. The early dates of collection and their distribution suggest Australian *C. melo* to be indigenous, perhaps dispersed in pre-European times by the aboriginal people as it is a wild food.

The last two Australian species, *C. variabilis* and *C. althaeoides*, represent another small 'radiation.' This limited diversification resembles the situation in other Cucurbitaceae colonizations of Australia (Schaefer et al. 2008, 2009). A recent review of vertebrate, invertebrate, and plant distribution ranges across the Australian monsoon tropics implicates the formation of the Carpentarian Gap, separating the Kimberley region and Arnhem Land from the Cape York Peninsula in the interruption of gene flow in numerous species (Bowman et al. 2009), and this event may also explain the *C. costatus* - *C. umbellatus* divergence (Fig. 1), which dates to the early Quaternary (Sebastian et al. 2010).

*Cucumis queenslandicus*, *C. costatus* and *C. althaeoides* inhabit savannah communities adapted to seasonal heavy rain and fire. They are perennials with thickened rootstocks allowing dormancy in the dry season and rapid growth in the wet. *Cucumis umbellatus* is an annual species occurring on the Kimberley - Arnhem Land sandstone plateau escarpments that are less fire-prone than savannah. *Cucumis variabilis* and *C. argenteus* inhabit some of the most arid parts of Australia and exhibit convergence vegetatively, both sometimes have leaves covered with dense, white, villous hairs (see the color photos of these species provided with their descriptions). *Cucumis picrocarpus* and the possible indigenous variant of *C. melo* grow on clay flats, such as alluvium on flood plains, but are recorded from a variety of habitats. More details about the vegetation types in which the Australian species occur are provided following the species descriptions.

#### TAXONOMIC TREATMENT

##### KEY TO SPECIES OF *CUCUMIS* IN AUSTRALIA AND EASTERN MALESIA

Identification of Australian *Cucumis* specimens requires flowers, fruits, and seeds; vegetative material could be identified by sequencing the ITS region and then BLASTing sequences against the complete *Cucumis* species sample in GenBank. The key below is applicable only to Australian material and includes both native and naturalized species, with the introduced African species marked by an asterisk.

- |  |                          |
|--|--------------------------|
| 1. Female flowers in fascicles, sometimes coxillary with several males   | 2                        |
| 2. Female flowers mostly 3-7 per axil; central Western Australia   | <i>C. variabilis</i>     |
| 2. Female flowers mostly 1 or 2, rarely 3 or 4 per axil; northern and inland Australia   | 3                        |
| 3. Seeds verrucose; mostly coastal E Malesia, northern Australia   | <i>C. althaeoides</i>    |
| 3. Seeds smooth; mostly inland Australia   | <i>C. argenteus</i>      |
| 1. Female flowers solitary   | 4                        |
| 4. Seeds pitted or verrucose   | 5                        |
| 5. Fruit globose or subglobose   | 6                        |
| 6. Fruit 1-3 seeded; fruiting pedicel > 5 cm long; male inflorescence umbellate  | <i>C. umbellatus</i>     |
| 6. Fruit 6-25-seeded; fruiting pedicel <1 cm long; male inflorescences fasciculate   | <i>C. althaeoides</i>    |
| 5. Fruit ellipsoidal, fusiform or ovoid-fusiform   | 7                        |
| 7. Male inflorescence racemose; fruit strongly ribbed, the ribs scabrid  | <i>C. costatus</i>       |
| 7. Male inflorescence fasciculate, rarely racemose; fruit weakly ribbed, the ribs hispid   | <i>C. queenslandicus</i> |
| 4. Seeds smooth  | 8                        |
| 8. Fruit aculeate; aculei scattered or dense, soft or rigid  | 9                        |
| 9. Fruit globose; aculei soft, scattered   | * <i>C. myriocarpus</i>  |
| 9. Fruit ellipsoidal   | 10                       |
| 10. Aculei dense, soft   | * <i>C. zeyheri</i>      |
| 10. Aculei scattered, rigidly pointed  | * <i>C. metuliferus</i>  |
| 8. Fruit smooth or longitudinally ribbed, variously hairy, sometimes glabrescent   | 11                       |
| 11. Leaves unlobed or shallowly 3- or 5-lobed; lobes < one third of lamina width; fruit with short antrorse hairs; native or naturalized | <i>C. melo</i>           |
| 11. Leaves deeply 5-lobed; lobes > one third of lamina width; fruit sparsely pilose with spreading hairs, glabrescent                    | <i>C. picrocarpus</i>    |

*CUCUMIS PICROCARPUS* F. Muell., descr. emend.

*Cucumis picrocarpus* F. Muell., Trans. Philos. Inst. Victoria 3: 46. 1859 as 'picrocarpa.'

*Cucumis picrocarpus* requires lectotypification because no specimens were cited in the protologue, only the distributional statement: "In many parts of tropical Australia" (von Mueller 1859). We carry out this lectotypification, together with another typification of a Mueller name, in an accompanying paper (I. Telford, et al. in prep.).

*Cucumis trigonus* auct. non Roxb.: Benth., Fl. Austral. 3: 317. 1863; F. M. Bailey, Queensland Fl. 696. 1900.

*Cucumis melo* auct. non L.: I. R. H. Telford, Fl. Australia 8: 189. 1982 p.p.; J. H. Kirkbride, Biosystematic monograph of the genus *Cucumis* (Cucurbitaceae), p. 79. 1993 p. p.

Trailing or climbing annual herb, monoecious, hispid on most vegetative parts; stems to 1.5 m long, ribbed. Tendrils simple, to 8 cm long. Leaves: petiole 30–123 mm long; lamina broadly ovate in outline, 28–140 × 26–130 mm, mucronate, deeply palmately 5-lobed, the lobes lobulate, denticulate. Inflorescences unisexual. Male flowers in (1)–3–8 flowered fascicles or racemes; peduncles to 4 mm long; pedicels 3.5–12(–35) mm long; hypanthium narrowly campanulate, 3.8–4.3 mm long, hispid with spreading hairs; calyx lobes linear to narrowly triangular, 1.8–2 mm long, hispid; corolla lobes elliptic or ovate, 18–22 × 10–12 mm, mucronate, sparsely hispid outside, particularly apically, glabrous inside, yellow; stamens inserted towards the base of the hypanthium tube; anthers one 1-theous, two 2-theous, sigmoid, ca. 2 mm long; connective appendage ca. 1 mm long, lobed, papillose; disc depressed globose, ca. 0.6 mm diam. Female flowers solitary; pedicels 2–25 mm long; ovary ellipsoidal, 4–6.5 mm long, 3–3.8 mm diam, pilose with spreading hairs; hypanthium above constriction 2–3.2 mm long; perianth similar to male; disc annular, ca. 1 mm diam, lobed; style 0.6–1 mm long; stigmatic branches 1.5–1.7 mm long. Fruits subglobose to ellipsoidal, 28–55 mm long, 25–40 mm diam, sparsely pilose with simple, multicellular hairs, glabrescent, pale green with darker longitudinal markings; fruiting pedicel to 56 mm long. Seeds many, elliptic, 4–5.6 × 2.2–3 mm, ± compressed with the faces slightly convex, the remnant of the funicle often remaining attached, appearing wing-like, smooth, buff.

**Representative Specimens Examined**—AUSTRALIA. Western Australia: 3.5 km WSW of Barrowana Hill, Hamersley Ranges, *Trudgen 18824* (PERTH); E side of old crossing at Fitzroy Crossing, *Mitchell 3070* (CANB, PERTH); Block 68, Sugar cane experimental area, Dept of Agriculture, Kununurra, *Aplin 6279* (K, PERTH); 100 m N of Lissadell Homestead main airstrip, 5 km NE of homestead, *Mitchell 3076* (CANB, PERTH). Northern Territory: Victoria Highway, ca. 19 km W from Victoria River crossing, *Purdie 3261* (CANB, NA); Sturts Creek North, Birrinbubu Station, *Maconochie 1753* (CANB, NT); Top Springs, *Telford 11659* (CANB, DNA, M, NE, NSW, US); Stuart Swamp, 5 km NNE of Daly Waters, *Latz 13729* (CANB, DNA); Rockhampton Downs, Government Paddock 7, *Wilson N402* (CANB, K, NT). Queensland: Camooweal Caves National Park, Nowranie Waterhole, *McDonald & Dennis 8916* (BRI, NE); Mount Eliza, 8.5 km along O'Briens Creek Road from Mount Surprise township, *Telford & Sebastian 13314* (M, NE), our Fig. 2A, B. New South Wales: Kirramingly Nature Reserve, ca. 30 km WSW of Moree, *Nano & M. Gardner NE96519* (NE); 'Burrenda', near Burren Junction, *Carrington CANB 310499* (CANB).

**Distribution**—Endemic to northern Australia from the Hamersley Ranges, Western Australia eastwards to Rockhampton, Queensland, and southwards in the Murray – Darling Basin to Burren Junction, New South Wales (Fig. 3).

**Habitat**—*Cucumis picrocarpus* grows in a variety of habitats, particularly on clay flats, such as alluvium on flood plains, but recorded also from rocky loam on hillsides. Vegetation communities include *Astrelba* grassland, grassy open woodland with *Eucalyptus*, *Bauhinia*, *Terminalia* and riverine woodland dominated by *Eucalyptus camaldulensis* Dehnh. and *E. microtheca* F. Muell.

**Phenology**—Flowers and fruits February–May.

**Conservation Status**—The species is widespread and common and is not considered at risk.

**Notes**—Von Mueller (1859) in the protologue described the fruits as extremely bitter. Several specimens in CANB and MEL were annotated "*Cucumis melo* subsp. nov." by Charles Jeffrey in 1986. *Cucumis picrocarpus* is sympatric with *C. melo* over much of its range (Fig. 3) but no intergrading or hybridization has been observed.

*CUCUMIS MELO* L., Sp. Pl. ed. 1, 1011. 1753.—TYPE: SWEDEN: Plant cultivated at Uppsala (lectotype: LINN, sheet number 1152.8, photograph!); see discussion in A. D. J. Meeuse (1962).

*Cucumis jucundus* F. Muell., Trans. Philos. Inst. Victoria 3: 46. 1859 as 'jucunda'.—TYPE: AUSTRALIA. Northern Territory: Victoria River, *Mueller s. n.* (neotype K, photograph!; isoneotype: K, photograph!), fide Kirkbride (1993).

*Cucumis pubescens* T. Mitch., J. Exped. Tropical Australia 110. 1848 nom. nud., non Willd. 1805.—TYPE: AUSTRALIA. 31 March 1848, latitude of camp, 28°38'47"S, *Mitchell s. n.* (holotype: K).

Trailing or climbing annual herb, hispid on most parts, monoecious; stems to 3 m long, 2.6 mm diam, ribbed. Tendrils simple, to 9 cm long. Leaves: petiole 15–80 mm long; lamina ovate in outline, 3–78 × 2.6–74 mm, unlobed or palmately shallowly 3- or 5-lobed, mucronate, denticulate. Inflorescences unisexual. Male flowers in (1)–3–5-flowered fascicles; pedicels 3–13 mm long; hypanthium narrowly campanulate, 3.5–4.3 mm long, pilose with spreading hairs; calyx lobes linear to narrowly triangular, 2–4 mm long; corolla lobes ovate to elliptic, 8–12 × 4–6 mm, mucronate, glabrous or sparsely hispid outside, glabrous inside, yellow; stamens inserted towards the base of the hypanthium tube; anthers one 1-theous, two 2-theous, sigmoid, ca. 2 mm long; connective appendage 1–2.2 mm long, lobed, papillose; disc depressed subglobose, ca. 0.8 mm diam. Female flowers solitary; pedicels 2.8–13 mm long; ovary ellipsoidal, 5–8 mm long, 2–3.4 mm diam, pilose with antrorse or spreading hairs; hypanthium above constriction 2.4–3 mm long; perianth similar to male; disc shortly cylindrical, ca. 1.3 mm diam, lobed; style 1–1.4 mm long; stigmatic branches 1.3–1.8 mm long. Fruit ellipsoidal, 20–36 mm long and 14–24 mm in diam, with short antrorse hairs, fruiting pedicel to 14 mm long. Seeds elliptic, 4.2–5 × 2–2.7 mm, ± compressed with the faces slightly convex, smooth, buff.

**Representative Specimens Examined**—AUSTRALIA. Western Australia: ca. 10 km W of Mount Vernon Station Homestead near Meekatharra, *Mitchell 4713* (CANB, PERTH); Hamersley Ranges National Park, on flats E of Mount Bruce, *Trudgen 2578* (PERTH), Fig. 2C shows an image of this specimen; Harding River bridge, Roebourne, *Olsson 57* (PERTH); ca. 100 km S of Dampier on Hamersley Iron Railway line road, *Mitchell PRP231* (CANB, PERTH); De Grey River, *Burbiidge 948* (PERTH). Northern Territory: Jasper Gorge, Victoria Downs road crossing of Jasper Creek, *Telford 11655* (BRI, CANB, DNA, NE, US). South Australia: Oodnadatta, *Knight 242* (AD); Lake Eyre basin, Scrubby Camp Waterhole, *Thorpe 85* (AD). Queensland: 47.9 km by road SW from Burke – Gulf Development

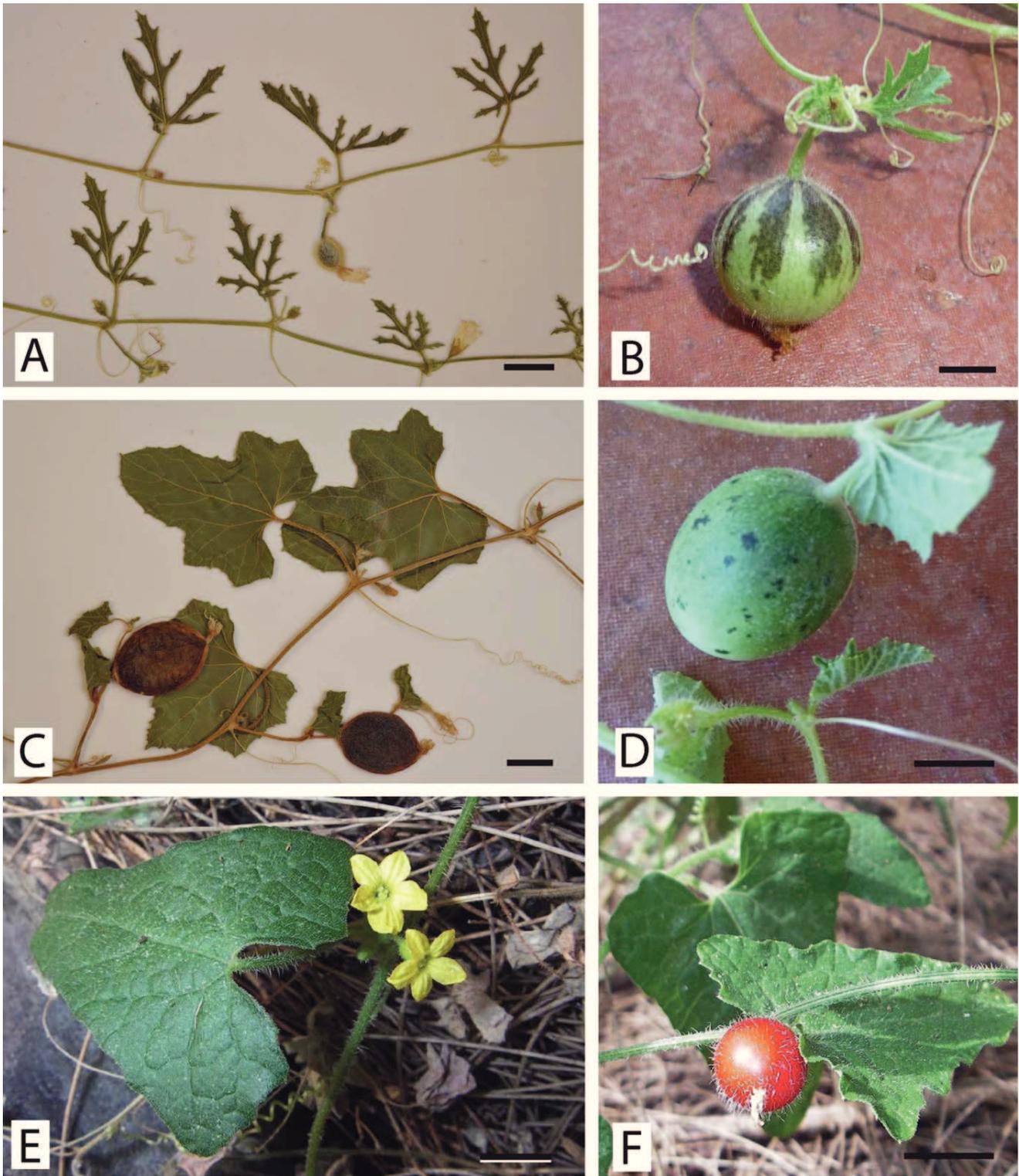


FIG. 2. Morphological traits of Australian *Cucumis* species. A, B. *Cucumis picrocarpus*. A. Flowering stems. B. Developing fruit (both from Telford & Sebastian 13314). C, D. *C. melo*. C. Flowering and fruiting stems (from Trudgen 2578); D. fruit (from Telford & Sebastian 13313). E, F. *C. althaeoides*. E. Male inflorescence. F. Fruiting stem. (both from Copeland & Bell 4220). Scale bars = 1 cm. Images A, C by J. J. Bruhl; B, D by P. Sebastian; E, F by L. M. Copeland.

Road junction near Normanton, McDonald & Dennis 8872 (BRI, NE); Mount Eliza, 8.5 km along O'Briens Creek Road from Mount Surprise township, Telford & Sebastian 13313 (M, NE), Fig. 2D shows an image of this collection; Long Hole, Winton Water Supply, Forster & Booth 22315 (BRI, DNA, MEL, NE).

**Distribution**—Widespread in Australia across the tropics and subtropics from near Meekatharra, Western Australia to Rockhampton, Queensland, south to Lake Eyre South, South Australia, and near Wilcannia, New South Wales (Fig. 3).

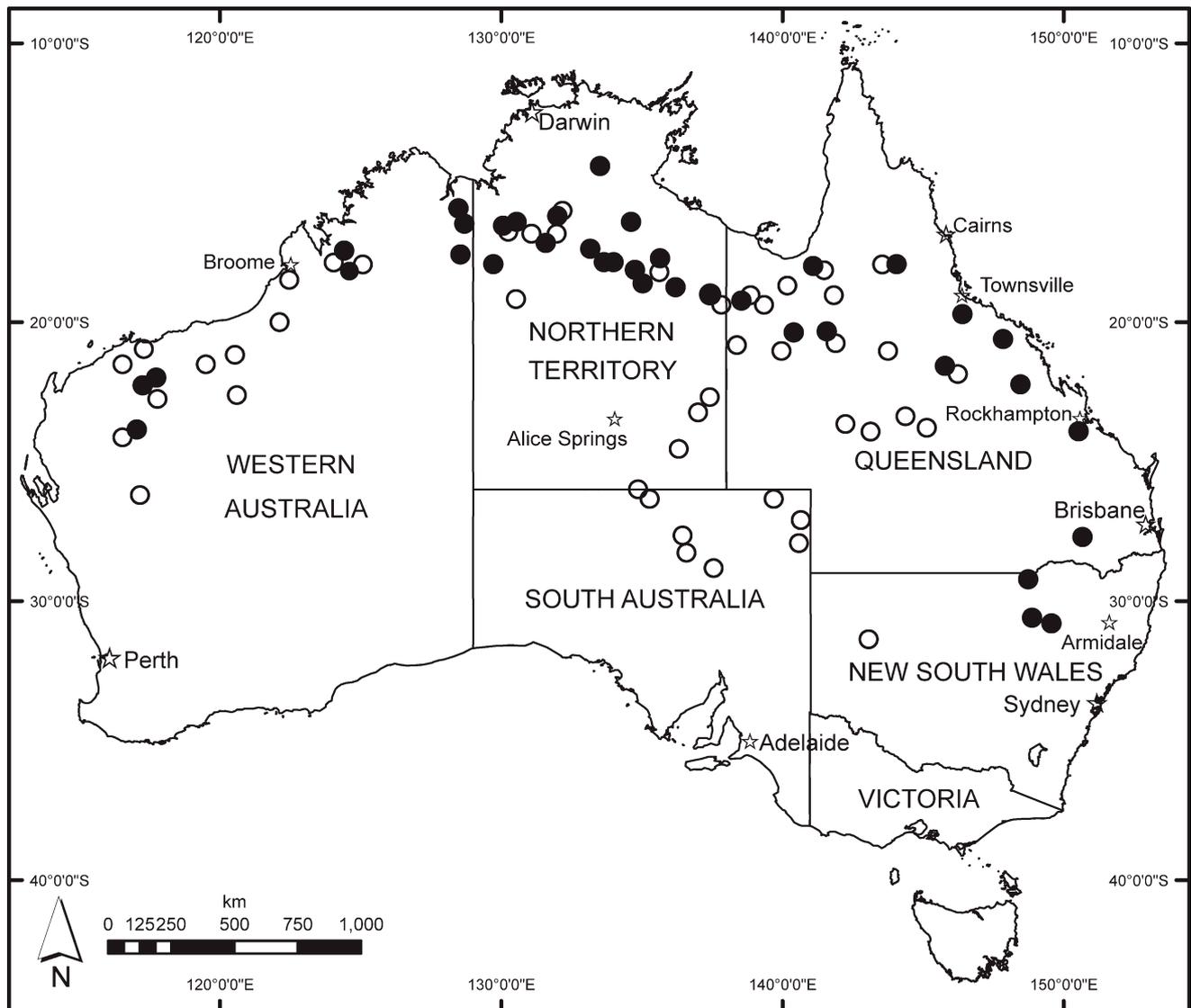


FIG. 3. Distributions of *Cucumis picrocarpus* (solid circle); *C. melo* (hollow circle).

**Habitat**—*Cucumis melo* grows in a variety of habitats including grasslands on cracking clays, *Eucalyptus*, *Corymbia*, *Acacia*, or *Grevillea* grassy woodlands on clay flats, less commonly on rocky slopes.

**Phenology**—Flowers and fruits January–May.

**Conservation Status**—The species is widespread and common and is not considered at risk. In Western Australia, the species is recorded as “common all along the Ashburton floodplain” (label data from Mitchell PRP231).

**Notes**—The description above is based on Australian material. This taxon was treated by Kirkbride (1993) as *Cucumis melo* L. subsp. *agrestis* (Naudin) Pangalo, a subspecies thought to occur throughout Africa, Asia, and Australia. The Australian populations appear to be indigenous as early European explorers already found *C. melo* in inland Australia before the establishment of Western settlements and gardens there. Because the fruits are wild food of aboriginal people, there is the possibility of anthropogenic range expansion, but resolving whether humans account for the species’ arrival in

Australia will require a detailed phylogeographic study of *C. melo*.

The aboriginal people in the Pilbara eat the fruits as a wild food (label data from Burbidge 948); the local aboriginal name is “Yindjibarndi” (label data from Olsson 57). In the Lake Eyre region, South Australia, “eaten by the Aborigines when falls off vine, rubbed in sand first”; local name “Ilcarta” (label data from Knight 242).

Plants of domestic cultivars are occasionally found as weeds around picnic areas (e.g. New South Wales: Namoi River, Warrabah National Park, Hosking 941) but they do not appear to persist.

***Cucumis costatus*** I. Telford sp. nov.—TYPE: AUSTRALIA. Queensland: Cook District: Mt Scatterbrain, Butchers Hill Station, near Lakeland, 15°52' S 144°53' E, 25 Jan. 1992, Forster 9514 (holotype: BRI!; isotypes: CNS!, MI!, MEL!, NE!).

*Mukia* sp. B, I. R. H. Telford, Fl. Australia 8: 186. 1982.

*Mukia racemosa* I. Telford, J. D. Briggs & J. Leigh, Rare or Threatened Australian Plants ed. 2: 44. 1995 nom. nud.

*Mukia* sp. (Little Annan R., *B. Gray 101*), R. J. F. Henderson (ed.), Names & Distributions of Queensland Plants 53. 2002; P. Bostock & A. E. Holland, Census of the Queensland Flora 54. 2007.

Ab affini *Cucumi javanico* differt floribus masculis in racemo digestis, fructu costato seminibusque turgidis.

Trailing or climbing herb, monoecious, hispid on most vegetative parts; stems to ca. 1.5 m long, ca. 1 mm diam, ribbed, sparsely hispid, annually sprouting from a thickened perennating rootstock. Tendrils simple, to 40 mm long. Leaves: petioles 10–33 mm long; lamina triangular to ovate, 25–35 × 18–28 mm, cordate, scarcely lobed or shallowly 3- or 5-lobed, dentate, the lobes obtuse, mucronate, hispid on both surfaces with hairs to 0.7 mm long. Inflorescences unisexual. Male flowers in 4–11-flowered racemes 25–37 mm long; peduncles 3–30 mm long; pedicels 3–12 mm long; hypanthium narrowly campanulate, 2.6–5.3 mm long, hispid; calyx lobes 5, narrow-triangular, 0.6–2 mm long; corolla lobes 5, rotate, ovate, 2.8–5.2 × 1–2.2 mm, apex rounded, glabrous or sparsely hispid outside, glabrous inside, yellow; stamens inserted about the middle of the hypanthium tube; anthers one 1-theous, two 2-theous, straight, ca. 2.2 mm long; connective appendage minute; disc depressed subglobose, ca. 1.3 mm diam. Female flowers solitary; peduncles 10–14 mm long, elongating in fruit; ovary narrowly fusiform, attenuate, 6–9.5 mm long ca. 2 mm diam, hispid with retrorse multicellular hairs; hypanthium above constriction ca. 3 mm long; perianth similar to male; staminodes absent; disc annular, ca. 1 mm diam; style ca. 1.8 mm long; stigma 3-branched, the branches ca. 1 mm long, each bifid for about half their length. Fruit fusiform or ellipsoidal, 16–32 mm long, 10–12 mm diam, longitudinally 9- or 10-ribbed, sparsely hispid, scabrid on ribs with retrorse tubercle-based hairs, green, 15–30-seeded; pericarp thin, showing seeds when dry; fruiting peduncle up to 40 mm long. Seeds ovate, 3.4–4 × 2.6–3 mm, pale brown, the faces convex, pitted, the margin narrow.

**Representative Specimens Examined**—AUSTRALIA. Queensland: McIvor River area, 0.5 km N of Tribulation Creek, 45 km NW of Cooktown, Wannan & Addicot 3845 (BRI); McIvor River, 5 km SW of Mt Ray, Wannan & Lyon 3931 (BRI, CNS, NE, NSW), our Fig. 4A and B; Endeavour River, Persieh 169 (MEL); Little Annan River crossing on the Cooktown road, Gray 101 (CNS); 0.9 km E of the West Normanby River on the Lakeland Downs to Cooktown road, Clarkson & MacDonald 6747 (BRI, CANB, CNS); northern ridge off Mount Sampson, Annan River catchment, McDonald et al. 2086 (BRI).

**Distribution**—Endemic to North-Eastern Queensland on the eastern fall of the Great Dividing Range in southern Cape York Peninsula, the species is recorded from the drainage basins of the McIvor, Endeavour, Annan, and Normanby Rivers (Fig. 5).

**Habitat**—*Cucumis costatus* grows in gallery forest or eucalypt woodland and depauperate deciduous vine thicket on low rocky hills, on basalt or metamorphics, to 300 m altitude. Associated species recorded include *Eucalyptus platyphylla* F. Muell., *E. leptophleba* F. Muell. and *Erythrophleum chlorostachys* (F. Muell.) Baillon.

**Phenology**—Flowers and fruits January–April.

**Conservation Status**—The species was allocated category 2K in Briggs and Leigh (1995), meaning it is a poorly known taxon with a geographic range of less than 100 km. At the type locality, it is recorded as “very common.” Additional recent collections indicate that *C. costatus* is more widespread than previously thought, and we therefore do not consider it at risk.

**Etymology**—From Latin *costatus* (ribbed), in reference to the pronounced longitudinal ribbing of the fruit.

**Notes**—*Cucumis costatus* was the first Australian *Cucumis* species sequenced for nuclear and chloroplast regions and is included in the phylogeny of Renner et al. (2007) as *Cucumis* sp. HS414. It differs from the morphologically similar *C. javanicus* in the male flowers being borne in racemes and costate fruits.

***Cucumis queenslandicus*** I. Telford sp. nov.—TYPE: AUSTRALIA. Queensland: Cook District: 5 km SE of Chillagoe, beside Burke Development Road, 18 Apr. 2006, Wannan & Beasley 4266 (holotype: BRI!; isotypes: CANB!, CNS!, M!, NE!).

Ab affini *Cucumi costato* differt foliis non profunde vel profunde lobatis, floribus masculis fasciculatis vel racemosis et fructu leviter costato.

Trailing or climbing perennial herb, monoecious, hispid on most vegetative parts; stems to 70 cm long, ribbed, annually sprouting from a thickened, perennating, ± cylindrical rootstock. Tendrils simple, to 60 mm long. Leaves: petioles (6–)14–40 mm long; lamina subtriangular to broadly ovate in outline, 9.5–44 × 11.5–42 mm, cordate, shallowly or deeply 3- or 5-lobed, when deeply lobed the lobes sinuately lobed, dentate, obtuse, mucronate, hispid on both surfaces with hairs to 0.2 mm long. Inflorescences unisexual. Male flowers in 2–5-flowered fascicles or racemes with peduncles to 12 mm long; pedicels 1.3–13 mm long; hypanthium narrowly campanulate, 2.8–4.2 mm long; calyx lobes 5, linear, 0.8–1.3 mm long; corolla lobes 5, obovate, 3.4–4.2 × 2–2.5 mm, rounded, glabrous or sparsely hispid outside, glabrous inside, yellow; stamens 3, inserted about the middle of the hypanthium tube; anthers one 1-theous, two 2-theous, straight, ca. 2 mm long; connective appendages minute; disc depressed globose, ca. 1.2 mm diam. Female flowers solitary; peduncle 5–18 mm long, scarcely elongating in fruit; ovary narrowly fusiform, ca. 5.5 mm long, ca. 1.2 mm diam, attenuate, densely hispid with retrorse, multicellular hairs; hypanthium above constriction ca. 6 mm long; perianth similar to male; disc annular, undulate, ca. 0.5 mm diam; style ca. 1.3 mm long; stigma capitate, ca. 2.5 mm long; staminodes absent. Fruit ovoid-fusiform, 15–23 mm long, 9–10 mm diam, slightly 10-ribbed, hispid, 10–20-seeded; pericarp thin, showing seeds when dry; fruiting pedicel 7–16 mm long. Seeds ovate, 4.5–5 × 3.8–4.2 mm, pale brown, the faces convex, pitted, the margin narrow. Figure 4C shows an image of the type collection.

**Representative Specimens Examined**—AUSTRALIA. Queensland: Cook District: Metal Hills section, Chillagoe – Mungana Caves National Park, McDonald 8814 (BRI, NE), Fig. 4D shows an image of this collection; Mt Eliza, 8 km NW of Mt Surprise, Forster & Bean 12812 (BRI); Mt Eliza, 8.5 km along O'Briens Creek Road from Mount Surprise township, Telford & Sebastian 13316 (BRI, CNS, M, NE); Undara National Park, Quartz Blow Lookout, McDonald 3662 (BRI); Newcastle Range, eastern flank, W of Mount Surprise, McDonald 3393 (BRI); near Copperfield River Dam, Kidston, Bean & Forster 7443 (BRI).

**Distribution**—*Cucumis queenslandicus* is endemic to North-Eastern Queensland on the western fall of the Great Dividing Range where it is known from near Chillagoe and from Undara Volcanic National Park to the Newcastle Range and south to the Copperfield River (Fig. 5).

**Habitat**—The species grows in *Eucalyptus* woodland on granite (Undara Volcanic National Park), the granite/limestone contact zone (Chillagoe), and deciduous vine thickets on granite hills (Mount Eliza and Undara Volcanic National Park) at 400–550 m altitude. Associated species recorded

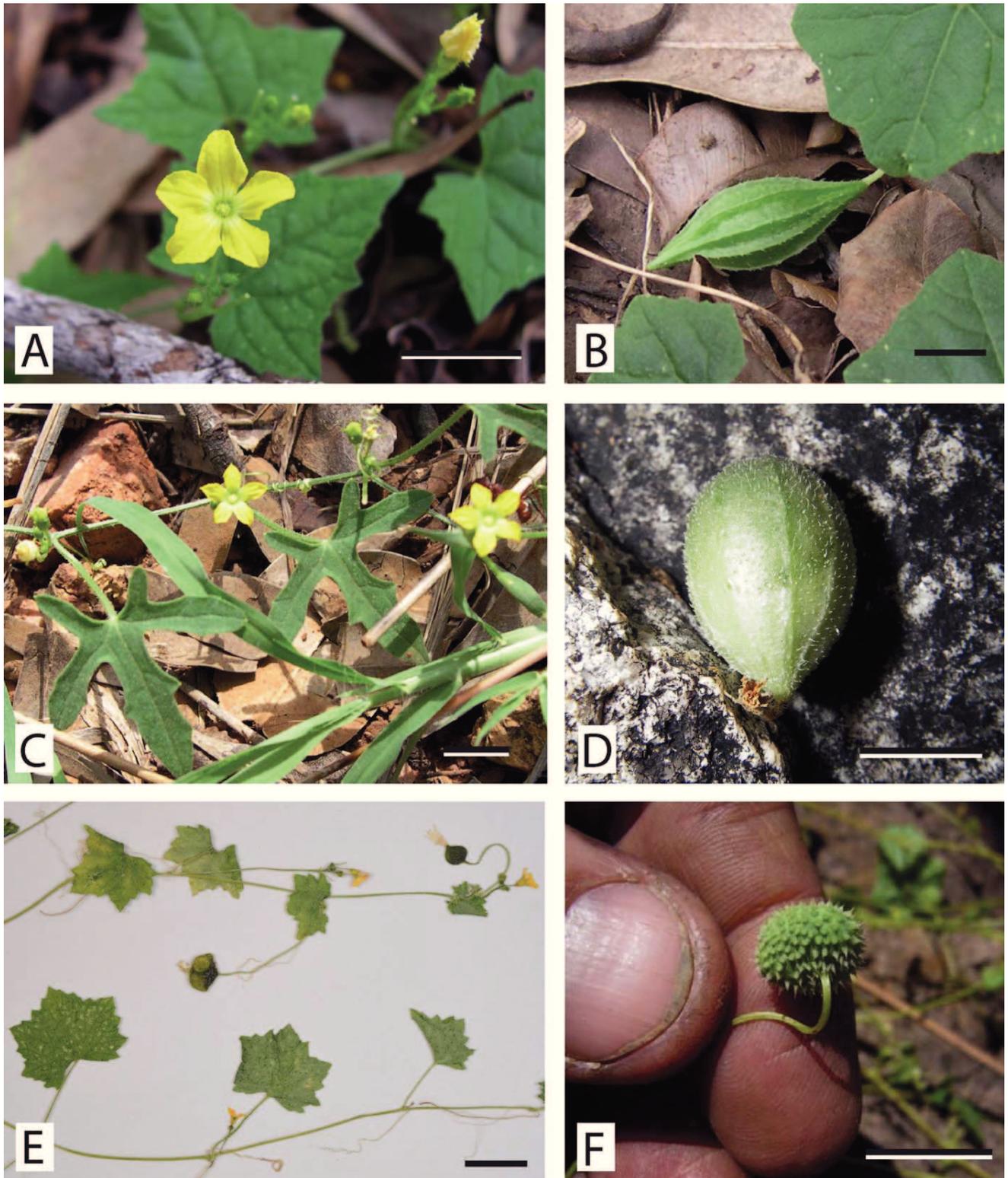


FIG. 4. Morphological traits of Australian *Cucumis* species. A, B. *Cucumis costatus*. A. Male inflorescences. B. Fruit (both from Wannan & Lyon 3931). C, D. *C. queenslandicus*. C. Male inflorescences (from Wannan & Beasley 4266). D. Fruit (from McDonald 8814). E, F. *C. umbellatus*. E. Flowering stems with developing fruit (from Sebastian 14). F. Fruit (from Wannan, Wardrop & Lane 5728). Scale bars = 1 cm. Images A–C, F by B. S. Wannan; D by K. R. McDonald; E by J. J. Bruhl.

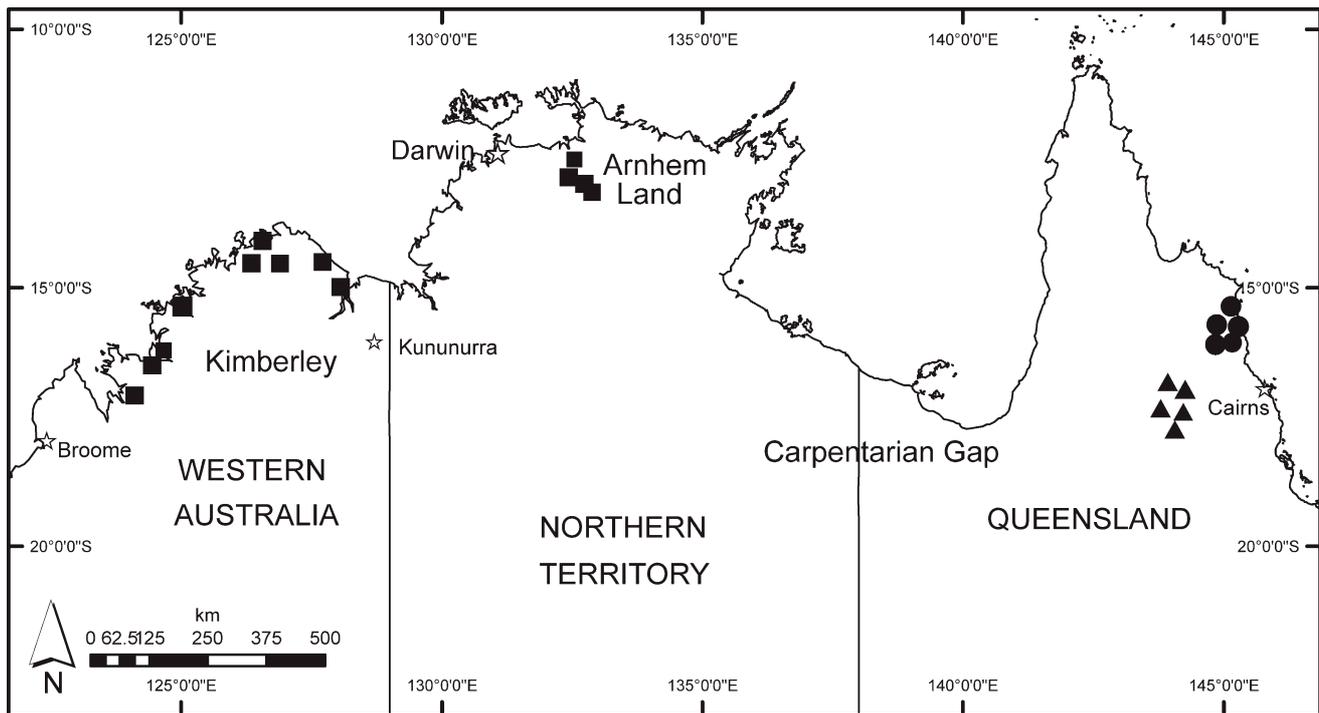


FIG. 5. Distributions of *Cucumis costatus* (circle); *C. queenslandicus* (triangle) *C. umbellatus* (square).

include *Eucalyptus cullenii* Cambage, *E. miniata* Schauer, *Erythrophloeum chlorostachys*, *Brachychiton chillagoensis* Guymmer and *B. albidus* Guymmer.

**Phenology**—Flowers and fruits January–June, mainly March–April.

**Conservation Status**—*Cucumis queenslandicus* is widespread with several recent collections in the Newcastle Range and Mount Surprise areas and is not considered at risk. It is conserved in Chillagoe – Mungana Caves and Undara Volcanic National Parks.

**Etymology**—Named for the state which includes the area of endemism of the species in north-eastern Queensland.

**Notes**—Molecular data (Fig. 1) corroborate an affinity to *C. costatus* and *C. umbellatus*. *Cucumis costatus* differs in having male flowers in more elongate racemes and strongly-ribbed fruit, *C. umbellatus* in umbellate male inflorescences and small unribbed fruit.

***Cucumis umbellatus*** I. Telford sp. nov.—TYPE: AUSTRALIA.

Northern Territory: Darwin and Gulf: Kakadu National Park, Ubirr, Apr. 2009, *Sebastian 14* (holotype: CANB!; isotypes, DNA!, K!, M!, MO!, NE!, PERTH!).

*Cucumis* sp. Gunlom (*J. L. McKean 864b*), Council of Heads of Australian Herbaria, Australian Plant Census. 2008 (<http://www.chah.gov.au/apc/index.html>)

*Mukia* sp. A, I. R. H. Telford, Fl. Australia 8: 184. 1982; J. R. Wheeler, Fl. Kimberley Region 252. 1992.

Ab affini *Cucumi costatus* differt floribus masculis umbellatis, fructibus longissime pedicellatis subglobosis vel ellipsoidalibus et seminibus paucioribus.

Trailing or climbing annual herb, monoecious, most vegetative parts sparsely hispid; stems to 2 m long, to 1 mm diam, ribbed. Tendrils simple, to 2 cm long. Leaves: petiole 8–35 mm long; lamina broadly ovate in outline, 10–40 × 14–45 mm,

cordate, shallowly 3- or 5-lobed, dentate, the lobes obtuse, mucronate, sparsely hispid on both surfaces with hairs to 1.8 mm long. Inflorescences unisexual. Male flowers in 12–20-flowered umbels; peduncles 10–55 mm long; pedicels 3–13 mm long; hypanthium campanulate, 1.6–2.2 mm long, sparsely hispidulous; calyx lobes linear, 0.5–0.8 mm long, sparsely hispidulous; corolla lobes ovate, 2.6–4 × 1.6–2 mm, obtuse, mucronate, glabrous or sparsely hispidulous outside, glabrous inside, yellow; stamens inserted about the middle of the hypanthium tube; anthers one 1-theous, two 2-theous, straight, ca. 1.2 mm long; connective appendages minute; disc depressed subglobose, ca. 0.7 mm diam. Female flowers solitary, rarely paired in axils; peduncle 5–25 mm long, greatly elongating in fruit; ovary subglobose to fusiform, ca. 0.8–1.2 mm diam, hispid with retrorse hairs; hypanthium above the constriction narrowly campanulate, 1.4–1.8 mm long; perianth similar to male; disc annular, ca. 0.6 mm diam; style ca. 0.8 mm long; stigmatic branches 0.8–1 mm long. Fruit subglobose, 4–7 mm diam, hispid with tubercle-based hairs, ripening bright orange, with 1 or 2, rarely 3 seeds; fruiting peduncle 50–115 mm long. Seeds ovate, 4.5–5 × 2.8–3.4 mm, buff, the faces convex, deeply pitted, the margin narrow. Figure 4E shows an image of the type specimen.

**Representative Specimens Examined**—AUSTRALIA. Western Australia: 15 km N of Mount Disaster, *Keighery 10601* (PERTH); Prince Regent River, *Wannan et al. 5728* (BRI, PERTH), Fig. 4F shows an image of this collection; island inside Yule Entrance, Walcott Inlet, *Mitchell 3531* (CANB, NE, PERTH); Boongarree Is., 18.3 km NE of Mount Knight, *Keighery 10695* (CANB, PERTH); mouth of Glenelg River, 20.5 km NW of Mount French, *Keighery 10689* (PERTH); tributary of Camp Creek, ca. 12 km SW of CRA Mining Camp, Mitchell Plateau, *Kenneally 7909* (PERTH); Carson Escarpment, 2.5 km N of Face Point, *Keighery 10667* (PERTH); Cambridge Gulf, 1887, *Wright* (MEL). Northern Territory: Obiri [Ubirr] Rock track, 4 km NW of Cahills Crossing, East Alligator River, *Telford & Wrigley 7631* (CANB, DNA, K, NE, PERTH); 1 mile SW of Cannon Hill, *Martenz & Schodde AE 647* (BRI, CANB, DNA, NT); Kakadu National Park, Gubarra, *Sebastian 15* (BRI, CANB, DNA, L, M, NE, US); Baroalba

Creek, ca. 1 km E of gorge entrance, *Cunliffe s. n.* (CANB, DNA, NSW, OSS, UNSW); Little Nourlangie Rock, *Telford & Wrigley 7814* (BISH, CANB); headwaters of Liverpool River, *Craven & Wightman 8363* (CANB).

**Distribution**—*Cucumis umbellatus* is endemic to northern Australia, occurring in the western and northern Kimberley region and disjunctly in the Northern Territory along the northwestern escarpment of Arnhem Land (Fig. 5).

**Habitat**—The species grows in sandy soils on sandstone, usually on rock outcrops or talus slopes or in rocky watercourses. The species occurs in vine thickets together with *Allosyncarpia ternata* S. T. Blake forest, in low open woodland with *Xanthostemon paradoxus* F. Muell. and *Terminalia hadleyana* W. Fitzg., and in open shrubland with *Triodia* species.

**Phenology**—Flowering and fruiting February–May.

**Conservation status**—*Cucumis umbellatus* is widespread in the Kimberley region, where it is conserved in Drysdale River National Park, and it is also common in Kakadu National Park. The species is therefore not considered at risk.

**Etymology**—From the Latin umbrella (parasol), in reference to the inflorescences of the male flowers.

**Notes**—The peduncle of the female flower elongates up to 115 mm as the fruit develops. This elongation may push the ripening fruit into the protection of crevices in the rocky slopes of its habitat. In the African *Cucumis humifructus*, the fruits are geocarpic; buried by the elongating pedicel (Meeuse, 1962). Although separated by a disjunction of some 500 km, no morphological differences between Kimberley and Top End populations are apparent.

**Cucumis argenteus** (Domin) P. Sebastian & I. Telford, comb. nov. *Melothria argentea* Domin, Biblioth. Bot. 89: 635, Fig. 196. 1929.—TYPE: AUSTRALIA. Queensland: Flinders River near the town Hughenden, Feb. 1910, K. Domin 8716 (holotype: PR, photograph!)

*Melothria celebica* var. *villosior* Cogn., Bull. Acad. Roy. Sci. Belgique ser. 3, 14: 357. 1887.—TYPE: AUSTRALIA. Gulf of Carpentaria, *Mueller s. n.* (holotype: BR photograph!; isotype: MEL!).

*Mukia maderaspatana* auct. non (L.) M. Roem.: I. R. H. Telford, Fl. Australia 8: 183, 185 Fig. 40E, G. 1982.

Trailing or climbing herb, monoecious, most vegetative parts pilose or densely hispid; stems to 3 m long, to 2 mm diam, ribbed. Tendrils simple, to 9 cm long. Leaves: sessile or petiole to 28 mm long; lamina triangular, lanceolate or broadly ovate in outline, 24–93 × 14–87 mm, cordate, acute or obtuse, mucronate, shallowly 3- or 5-lobed, denticulate, densely scabrid or villous with hairs to 1.2 mm long on both surfaces. Inflorescences unisexual. Male flowers in 2–5-flowered fascicles; pedicels to 7 mm long; hypanthium campanulate, 3–3.5 mm long, pilose or hispid; calyx lobes linear, ca. 1 mm long, pilose or hispid; corolla lobes ovate, 3.4–3.6 × 2.5–2.7 mm, rounded, sparsely pilose or puberulous outside, the hairs denser apically, puberulous inside, pale yellow; stamens inserted about the middle of the hypanthium tube; anthers one 1-theous, two 2-theous, straight, ca. 2 mm long; connective appendages minute; disc depressed subglobose, 0.7–1.3 mm diam. Female flowers 1 or 2 per axil, sometimes axillary with several males; sessile or pedicels to 2 mm long; ovary subglobose, ca. 1.5 mm diam, white pilose with antrorse hairs; hypanthium above the constriction narrowly campanulate, 2.4–3 mm long; perianth similar to male; disc annular, 1–1.5 mm diam; style ca. 1.8 mm long; stigmatic branches ca. 1.8 mm long. Fruits globose, 6.4–8 mm diam, pale green with

darker longitudinal markings, at maturity sparsely or densely pilose, red, with 5 seeds; fruiting pedicel to 2.5 mm long. Seeds ellipsoidal, 4.5–4.8 × 2.8–3.3 mm, the faces convex, smooth, lacking a thickened margin, grey-black or pale brown.

**Representative Specimens Examined**—AUSTRALIA. Western Australia: 246 km from Broome along Great Northern Highway towards Port Hedland, *Telford & Butler 6028* (BISH, CANB, K, MO, PERTH); Wolf Creek Crater, *George 15338* (NT, PERTH). Northern Territory: Petermann Ranges, Lasseters Cave, *Henshall 3450* (AD, DNA, NT); Kings Canyon National Park, *Bruhl & Quinn 2162* (L, NE); Ilparpa Road, Alice Springs, *Albrecht 10202* (CANB, NT), Fig. 6B shows an image of this specimen; West Island, Sir Edward Pellew Group, *Braithwaite 3290* (CANB). South Australia: Lake Eyre region, Peake Creek, *Conrick 724* (AD, CANB). Queensland: Riversleigh archaeological site D, Australian Geographic information area, *Barnsley 1656* (BRI, CANB, NE), Fig. 6A shows an image of this specimen; 20.2 km by road S of Musselbrook Mining Camp on road to Camooweal, *Thomas & Johnson s. n.* (A, AD, BRI, DNA, K, NE); 3 km from Mount Isa, *Ollerenshaw & Kratzing 1161* (BRI, CANB, L).

**Distribution**—Endemic to Australia, *Cucumis argenteus* occurs widely from Ninety Mile Beach, Western Australia, southwards to the Flinders Ranges, South Australia, eastwards to Winton, Queensland (Fig. 7).

**Habitat**—*Cucumis argenteus* grows in a range of habitats from sand plains, loamy flats, rocky hillsides and limestone ridges to tussock grassland, *Triodia* hummock grassland, open shrublands and eucalypt or *Acacia* woodland.

**Phenology**—Flowers and fruits throughout the year, possibly in response to rainfall, but mostly between April and August in tropical areas.

**Notes**—Three collections by K. Domin of *Melothria argentea* from the Flinders River near Hughenden, Queensland, are held in PR, numbered *Domin 8715*, *1816*, and *8717*. Although no collector's number is given in the protologue, the image is of *K. Domin 1816*, with the caption "*Melothria argentea* Dom. nach dem Originalexemplare von Flinders River frequenter." The species differs from *C. rumphianus* in its smaller fruit and seeds. Being widely distributed, the species is accordingly variable in leaf size and indumentum length and density. Collections from the Barkly Tableland and its northern slopes, Queensland, are densely white hairy; plants further inland are scabrid. Specimens with the dense, whitish indumentum only on the lower leaf surface have been collected (*Braithwaite 3290*, *Ollerenshaw & Kratzing 1161*). Further study may lead to the recognition of two subspecies.

**Cucumis althaeoides** (Ser.) P. Sebastian & I. Telford, comb. nov. *Bryonia althaeoides* Ser. in DC. Prodr. 3: 306. 1828; *Mukia althaeoides* (Ser.) M. Roem. Syn. Monogr. 2: 47. 1846; *Melothria althaeoides* (Ser.) Nakai, J. Jap. Bot. 14: 127. 1938 (as "*althaeoides*").—TYPE: TIMOR. *leg. ign.* (holotype: G-DC, photograph!).

*Mukia maderaspatana* auct. non (L.) M. Roem.: I. R. H. Telford, Fl. Australia 8: 183, 185 Fig. 40F. 1982; W. J. J. O. de Wilde & B. E. E. Duyfjes, Thai For. Bull. (Bot.) 34: 43.2006 p. p.

Trailing or climbing perennial herb, monoecious, most vegetative parts hispid, sometimes pilose; stems to 3 m long, to 1.6 mm diam, ribbed, annually sprouting from a perennating rootstock. Tendrils simple, to 15 cm long. Leaves: sessile or petiole to 38 mm long; lamina ovate or broadly lanceolate in outline, sometimes hastate, 24–75 × 18–70 mm, cordate, unlobed or shallowly 3-lobed, rarely 5-lobed, obtuse or acute, mucronate. Inflorescences unisexual. Male flowers in 3–10(–15)-flowered fascicles, sometimes in racemes with peduncles to 3 mm long; pedicels to 7 mm long; hypanthium narrowly campanulate, 3–3.4 mm long, hispid or pilose

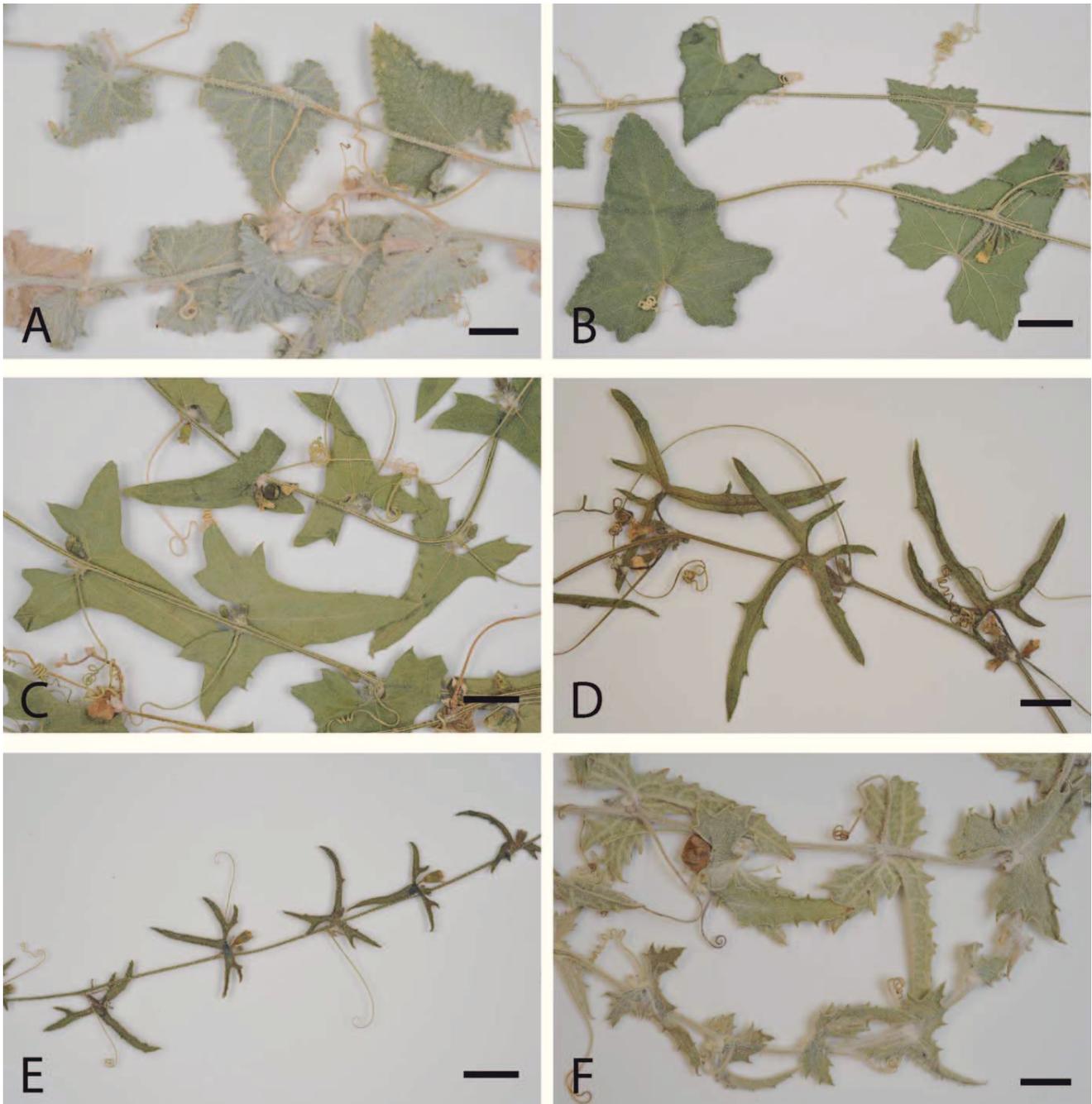


FIG. 6. Morphological traits of Australian *Cucumis* species. A, B. *Cucumis argenteus*. A. Typical specimen from Barkly Tableland, Queensland (Barnsley 1656). B. Typical specimen from Central Australia (Albrecht 10202). C–F. *C. variabilis*. C. Typical specimen from southern Pilbara, Western Australia (from Byrne 2809). D. Variant from North West Cape, Western Australia (from Wajon 473). E. Variant from Barrow Island, Western Australia (from White 87). F. Pilbara variant (from Payne PRP 1864). Scale bars = 1 cm. Images by J. J. Bruhl.

outside; calyx lobes linear, 0.8–1.4 mm long, hispid or pilose; corolla lobes ovate, 3.8–4.2 × 2.6–2.8 mm, rounded, mucronate, sparsely hispid outside, the hairs denser apically, glabrous or hispid along the veins inside, hispid around the mouth of the hypanthium tube, bright yellow; stamens inserted about the middle of the hypanthium tube; filaments ca. 0.4 mm long; anthers one 1-theous, two 2-theous, straight, 1.5–2 mm long; connective appendages minute; disc depressed globose, 0.8–1.4 mm diam. Female flowers 1 or 2, rarely 3 or 4 per axil; pedicels to 2 mm long; ovary ellipsoidal, ca. 3 mm long,

pilose with antrorse hairs; hypanthium above the constriction narrowly campanulate, ca. 3 mm long; perianth similar to male; disc annular, 1–1.2 mm diam; style ca. 1.5 mm long; stigmatic lobes 3, ca. 1.3 mm long. Fruit globose, 8–18 mm diam, pale green with darker longitudinal markings, at maturity sparsely pilose, red, with 9–20(–25) seeds; fruiting pedicel to 6 mm long. Seeds ovate, 3.8–4.5 × 2.3–2.8 mm, buff, the faces convex, verrucose, the margin thickened, raised.

*Representative Specimens Examined*—INDONESIA: Timor, Zippel 107 (L). PAPUA NEW GUINEA: near Matapaili village, Darbyshire 696

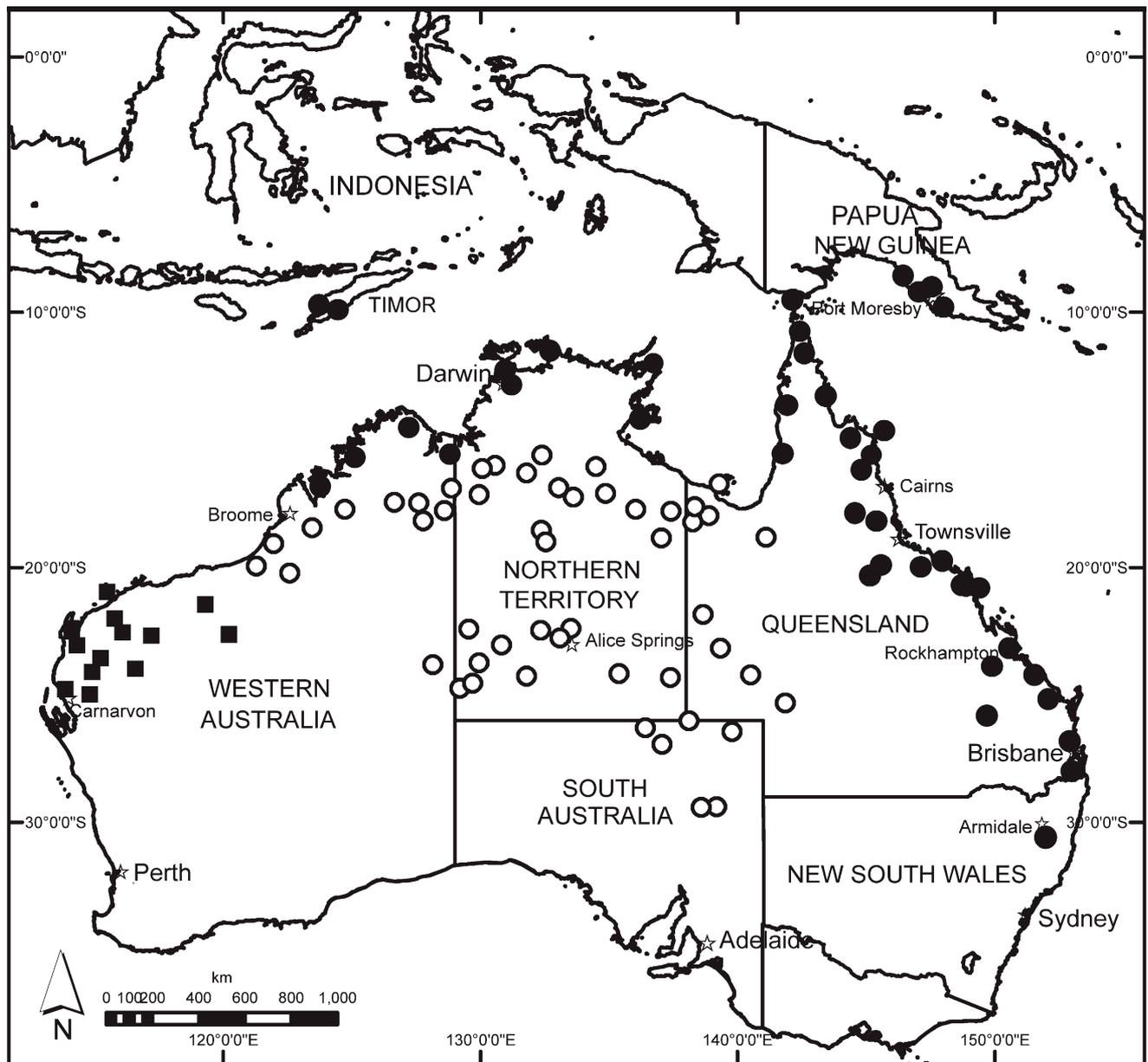


FIG. 7. Distributions of *Cucumis althaeoides* (solid circle); *C. argenteus* (hollow circle); *C. variabilis* (square).

(A, CANB, L, LAE); S coast near Hood Bay, *Pajmans* 775 (CANB); SE side of Little Mount Lawes, ca. 16 miles N of Port Moresby, *Pullen* 6804 (A, BRI, CANB, L, LAE, K, TNS). AUSTRALIA: Western Australia: Glycosmis Bay, *Mitchell* 7726 (PERTH). Northern Territory: Humpty Doo, *McKee* 8320 (DNA); 13 km SW of Cape Arnhem, *Brennan* 2576 (DNA). Queensland: Lizard Island, Mangrove Beach, *Batianoff & Tarte* 12083 (A, BRI, DNA, K, LE); McIvor River, "Mount Ray", 1.5 km NW of Mount Ray, *Telford & Sebastian* 13308 (BRI, CNS, L, M, NE); Bucasia Beach, 13 km N of Mackay, *Clarke* NE 81288 (CANB, M, NE); Tannum Sands, 15 km SE of Gladstone, *Telford* 5499 (CANB). New South Wales: Oxley Wild Rivers National Park, Aspley River, 300 m downstream from Rusdens Creek, *Copeland & Bell* 4220 (CANB, M, NE, NSW), our Fig. 2E and F.

**Distribution**—*Cucumis althaeoides* occurs in coastal and sub-coastal Timor, southern New Guinea and northern Australia, from the Kimberley region in Western Australia eastwards through the Northern Territory, Queensland and south to near Walcha, New South Wales (Fig. 7).

Nakai (1938) cites collections from the Ryukyu Islands and Taiwan, and states that the species also occurs on the

Philippines, Borneo, New Guinea, and Timor (besides Australia). As yet, we have not studied specimens from north of Timor.

**Habitat**—The species grows on coastal sands or riverine alluvium in herbfields and *Casuarina* or *Eucalyptus* woodland. On coralline beach sands of eastern Queensland, the species is recorded as growing under *Casuarina equisetifolia* L. and *Pandanus tectorius* Parkinson ex J. P. du Roi.

**Phenology**—Flowers and fruits April–August.

**Conservation Status**—Widespread and common, the species is not considered at risk.

**Notes**—*Cucumis althaeoides* differs from other Australian species in its verrucose seeds with thickened margins. Why Nakai (1938) treated the species under *Melothria* is unclear to us. The species shows considerable variation in leaf morphology over its range, notably in petiole length and degree of lobing of the lamina. In southern Papua New Guinea and northeastern Queensland, plants bearing larger,

many-seeded fruits (up to 1.8 mm diam long, with 16–20 seeds) occur and perhaps warrant formal recognition at the rank of subspecies.

Besides *C. althaeoides*, a second species [*A. R. Insani SAN 10* (L), collector ignotus 1305 (L), *H. Raap 499* (L), the latter in our Fig. 1] occurs on Timor and westwards to at least Java. Based on the molecular data, this still unnamed species is sister to *C. althaeoides* and *C. variabilis*.

***Cucumis variabilis*** P. Sebastian & I. Telford sp. nov.—TYPE: AUSTRALIA. Western Australia: 46.5 km along North West Coastal Highway from Ashburton River bridge towards Carnarvon, 25 Apr. 1992, *Telford 11578* (holo: PERTH!; iso: BRI!, CANB!, K!).

*Mukia maderaspatana* auct. non (L.) M. Roem.: I. R. H. Telford, Fl. Australia 8: 183. 1982.

Ab affini *Cucumi althaeoidi* differt foliis non profunde vel profunde lobatis, lobis plerumque angustissimis, seminibusque laevibus margine carentibus.

Trailing or climbing herb, monoecious, most vegetative parts hispid or pilose; stems to 2 m long, to 1.8 mm diam, ribbed. Tendrils simple, to 13 cm long. Leaves: petiole 2.4–18 mm long; lamina triangular, lanceolate or ovate in outline, often hastate or sagittate, 16–102 × 14–55 mm, cordate, acuminate, mucronate, shallowly to deeply 3- or 5-lobed, usually sparsely serrate, on deeply lobed leaves, the lobes usually narrow, linear. Inflorescences mostly unisexual. Male flowers in 5–14-flowered fascicles, sometimes in racemes on peduncles to 4 mm long; pedicels 3–7.5(–12) mm long, at first pilose; hypanthium campanulate, 3.5–4.2 mm long; calyx lobes linear, 1–1.3 mm long, hispidulous outside; corolla lobes ovate, 4.6–5 × 3–3.4 mm, obtuse, mucronate, hispidulous outside, glabrous inside, yellow; stamens inserted about the middle of the hypanthium tube; filaments 0.6–1.2 mm long; anthers one 1-theous, two 2-theous, straight, 2.2–2.6 mm long; connective narrow; appendages minute; disc depressed subglobose, ca. 1.2 mm diam. Female flowers in 3–7-flowered fascicles; subsessile or pedicels to 8 mm long; ovary subglobose, ca. 1.8 mm diam, hispidulous with antrorse hairs; hypanthium above the constriction narrowly campanulate, ca. 4 mm long; perianth similar to male; disc annular, ca. 1.4 mm diam, style ca. 2 mm long; stigmatic branches 0.8–1.2 mm long. Fruit globose, 5–8 mm diam, at maturity glabrescent, ripening orange-red, with 10–15 seeds; fruiting peduncle to 8.5 mm long. Seeds ovate, 5.6–6.5 × 3–3.4 mm, the faces convex, smooth, minutely verrucose in two lines around the nonprojecting margin, grey or pale brown.

**Representative Specimens Examined**—AUSTRALIA. Western Australia: Barrow Island, 75 m E of turnoff to R33, *White 87* (PERTH), Fig. 6E shows an image of this specimen; Yardie Creek Road, Cape Range National Park, near Exmouth, *Wajon 473* (PERTH), Fig. 6D shows an image of this specimen; 3 miles N of Exmouth, *Allan 469* (CANB, MEL, PERTH); Carawine Pool, ca. 140 km SE of Shay Gap, *Newbey 10312* (PERTH); Cape Range, road to nos 3 and 4 wells, *George 2470* (PERTH); ca. 25 km N of Marble Bar on small track W of Shay Gap road, *Mitchell PRP905* (CANB, PERTH); Shellborough, 40 km NNW of Goldsworthy, *Telford & Butler 6476* (CANB, PERTH); coast NW of Ilgarene Well, De Gray Station, *Payne PRP1864* (PERTH), Fig. 6F shows an image of this specimen; ca. 23.4 km WNW of De Grey Homestead, adjacent to Freshwater Bore, *Mitchell PRP868* (CANB, PERTH); Roy Hill, *Byrne 2809* (PERTH), Fig. 6C shows an image of this specimen; Barrabiddy Creek, *Ashby 2944* (PERTH); 45 km E of Carnarvon, *Wilson 8389* (CANB, L, PERTH); 10 km ENE of West Angela Hill, Hamersley Ranges, *Trudgen 15877* (PERTH); 11.6 km WSW of Mount Sabine, Millstream-Chichester National Park, Hamersley Ranges, *Weston 20385* (PERTH).

**Distribution**—*Cucumis variabilis* is endemic to Western Australia, where it ranges from NE of Onslow to the Cape

Range and Barrow Island, southwards to Carnarvon and the Ashburton River and eastwards to NE of Nullagine (Fig. 7).

**Habitat**—The species grows on calcareous or siliceous sand and on rocky ridges and slopes in *Eucalyptus* low open woodland, *Acacia* shrubland and *Triodia* hummock grassland.

**Phenology**—Flowers and fruits May–October.

**Conservation Status**—*Cucumis variabilis* is widespread and not considered at risk. It is conserved in Cape Range, Hamersley Ranges, and Millstream-Chichester National Parks.

**Etymology**—Latin *variabilis* (varying), in reference to the vegetative variability of the species.

**Notes**—*Cucumis variabilis* has been illustrated in Telford (1982: Figs. 40G, 40E), both times as *Mukia maderaspatana*, showing the extremes of leaf variability. The species shows two lines of variation: leaf morphology and indumentum. More southerly populations inland from Carnarvon bear larger leaves with a scattered, scabrid indumentum. Populations from North West Cape have a similar indumentum but narrower leaf segments with extreme reduction shown in the Barrow Island population. This island population has been listed as a putative new species, “*Cucumis* sp. Barrow Island” (*D. W. Goodall 1264*), in the Western Australian “FloraBase” (<http://florabase.calm.wa.gov.au/>). In the Pilbara, plants bear thicker leaves with a dense, white, villous indumentum similar to Barkly Tableland populations of *C. argenteus*. These variants may eventually deserve formal taxonomic recognition as subspecies.

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#### LITERATURE CITED

- Bowman, D. M. J. S., G. K. Brown, M. F. Braby, J. R. Brown, L. G. Cook, M. D. Crisp, F. Ford, S. Haberle, J. Hughes, Y. Isagi, L. Joseph, J. McBride, G. Nelson, and P. Y. Ladiges. 2009. Biogeography of the Australian monsoon tropics. *Journal of Biogeography* 37: 201–216.
- Briggs, J. D. and J. H. Leigh. 1995. Rare or threatened Australian plants. Melbourne: CSIRO Publishing.
- De Wilde, W. J. O. and B. E. E. Duyfjes. 2006. *Mukia* Arn. (Cucurbitaceae) in Asia, in particular in Thailand. *Thai Forest Bulletin (Botany)* 34: 38–52.
- Felsenstein, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Zoology* 22: 240–249.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Ghebretinsae, A. G., M. Thulin, and J. C. Barber. 2007a. Nomenclatural changes in *Cucumis* (Cucurbitaceae). *Novon* 17: 176–178.
- Ghebretinsae, A. G., M. Thulin, and J. C. Barber. 2007b. Relationships of cucumbers and melons unraveled: Molecular phylogenetics of *Cucumis* and related genera (Benincaseae, Cucurbitaceae). *American Journal of Botany* 94: 1256–1266.
- Kirkbride, J. H. 1993. Biosystematic monograph of the genus *Cucumis* (Cucurbitaceae). Boone, North Carolina: Parkway Publishers.
- Kocyan, A., L.-B. Zhang, H. Schaefer, and S. S. Renner. 2007. A multi-locus chloroplast phylogeny of the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution* 44: 553–577.
- Meuse, A. D. J. 1962. The Cucurbitaceae of Southern Africa. *Bothalia* 8: 1–111.

- von Mueller, F. 1859. Some hitherto unknown Australian Plants. *Transactions of the Philosophical Institute of Victoria* 3: 46.
- Nakai, T. 1938. On the Japanese species of *Melothria*. *Journal of Japanese Botany* 14: 122–131.
- Renner, S. S. and H. Schaefer. 2008. Phylogenetics of *Cucumis* (Cucurbitaceae) as understood in 2008. Pp. 53–58 in *Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae Avignon, France 21–24 May, 2008*, ed. M. Pitrat. Avignon: INRA.
- Renner, S. S., H. Schaefer, and A. Kocyan. 2007. Phylogenetic relationships among *Cucumis* (Cucurbitaceae): Cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evolutionary Biology* 7: 58.
- Roemer, M. 1846. *Familiarum Naturalium Regni Vegetabilis Synopses Monographicae seu Enumeratio Omnium Plantarum hucusque Detectarum Secundum Ordines Naturales, Genera et Species Digestarum, Additas Diagnosibus, Synonymis, Novarumque vel Minus Cognitarum Descriptionibus Curante M. J. Roemer*. Vol. 2. Weimar: Landes Industrie Comptoir.
- Schaefer, H. 2007. *Cucumis* (Cucurbitaceae) must include *Cucumella*, *Dicoelospermum*, *Mukia*, *Myrmecosicyos* and *Oreosyce*: a recircumscription based on nuclear and plastid DNA data. *Blumea* 52: 165–177.
- Schaefer, H., I. R. H. Telford, and S. S. Renner. 2008. *Austrobryonia* (Cucurbitaceae), a new Australian endemic genus, is the closest living relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*. *Systematic Botany* 33: 125–132.
- Schaefer, H., C. Heibl, and S. S. Renner. 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings. Biological Sciences* 276: 843–851.
- Sebastian, P., H. Schaefer, I. R. H. Telford, and S. S. Renner. 2010. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences USA* 107: 14269–14273.
- Seringe, N. C. 1828. Cucurbitaceae. In A. P. De Candolle, ed., *Systematis Naturalis Regni Vegetabilis* 3: 297–320.
- Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75: 758–771.
- Telford, I. R. H. 1982. Cucurbitaceae. Pp. 158–198 in *Flora of Australia*, ed. A. S. George, Canberra: A. S. Australian Government Publishing Service.

## CHAPTER 3

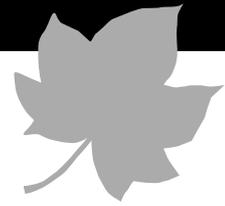
### DARWIN'S GALAPAGOS GOURD: PROVIDING NEW INSIGHTS 175 YEARS AFTER HIS VISIT

SEBASTIAN, P., SCHAEFER, H. AND RENNER, S.S. 2010.

*Journal of Biogeography*, 37: 975-980.



CORRESPONDENCE



**Darwin's Galapagos gourd: providing new insights 175 years after his visit**

**ABSTRACT**

The year 2010 marks the 175th anniversary of Charles Darwin's visit to the Galapagos Islands. A recent paper by J. C. Briggs, 'Darwin's biogeography' (*Journal of Biogeography*, 2009, 36, 1011–1017), summarizes Darwin's contributions to the field of biogeography, stressing the importance of his natural history specimens. Here, we illustrate how a plant collected by Darwin during his visit to Floreana and not collected since can provide insights into dispersal to oceanic islands as well as extinction of island plants, based on ancient DNA from Darwin's herbarium specimen.

**Keywords** Cucurbitaceae, Darwin's herbarium, extinction, Galapagos, island biogeography, molecular clock, phylogenetics, plant viruses, *Sicyos*.

During his voyage on the *Beagle*, Charles Darwin industriously collected natural history specimens and sent them home from various ports along the way (Briggs, 2009). On his return to Great Britain, he immediately began supervising work on the scientific results of the expedition, employing the help of specialists to record his fossils, fishes, mammals, birds and plants. Among the 209 plants that Darwin collected on the Galapagos Islands and sent to J. D. Hooker was a new species of Cucurbitaceae, *Sicyos villosus* J. D. Hooker (Fig. 1). Unlike his bird collections, Darwin labelled his plants by island, which is why we know that this cucurbit came from Charles Island, now Floreana. On the herbarium label, the species is described as 'In great beds injurious to vegetation.' Since then, this species has never been found again, despite intense search efforts of several botanists. Based on one fruit from a bag attached to Darwin's 175-year-old specimen in the Cambridge

herbarium (Fig. 1, insert), one of us (H.S.) generated nuclear and chloroplast sequences to find out the affinities of this vanished mystery plant. We also tested its relationship to another endemic Galapagos cucurbit, the Santa Cruz gourd, *Sicyocaulis pentagonus* Wiggins, which is known from five collections on Santa Cruz and Isabela, but has not been collected in the past 35 years.

Based on molecular data (Kocyan *et al.*, 2007; Schaefer *et al.*, 2008, 2009), the New World Sicyoeae comprise a clade of about 150 species in 20 genera (*Apatzingania*, *Brandegea*, *Cyclanthera*, *Echinocystis*, *Echinopepon*, *Elateriopsis*, *Frantzia*, *Hanburia*, *Linnaeosicyos*, *Marah*, *Microsechium*, *Parasicyos*, *Pseudocyclanthera*, *Rytidostylis*, *Sechiopsis*, *Sechium*, *Sicyocaulis*, *Sicyos*, *Sicyosperma*, *Vaseyanthus*). Herbarium specimens of 76



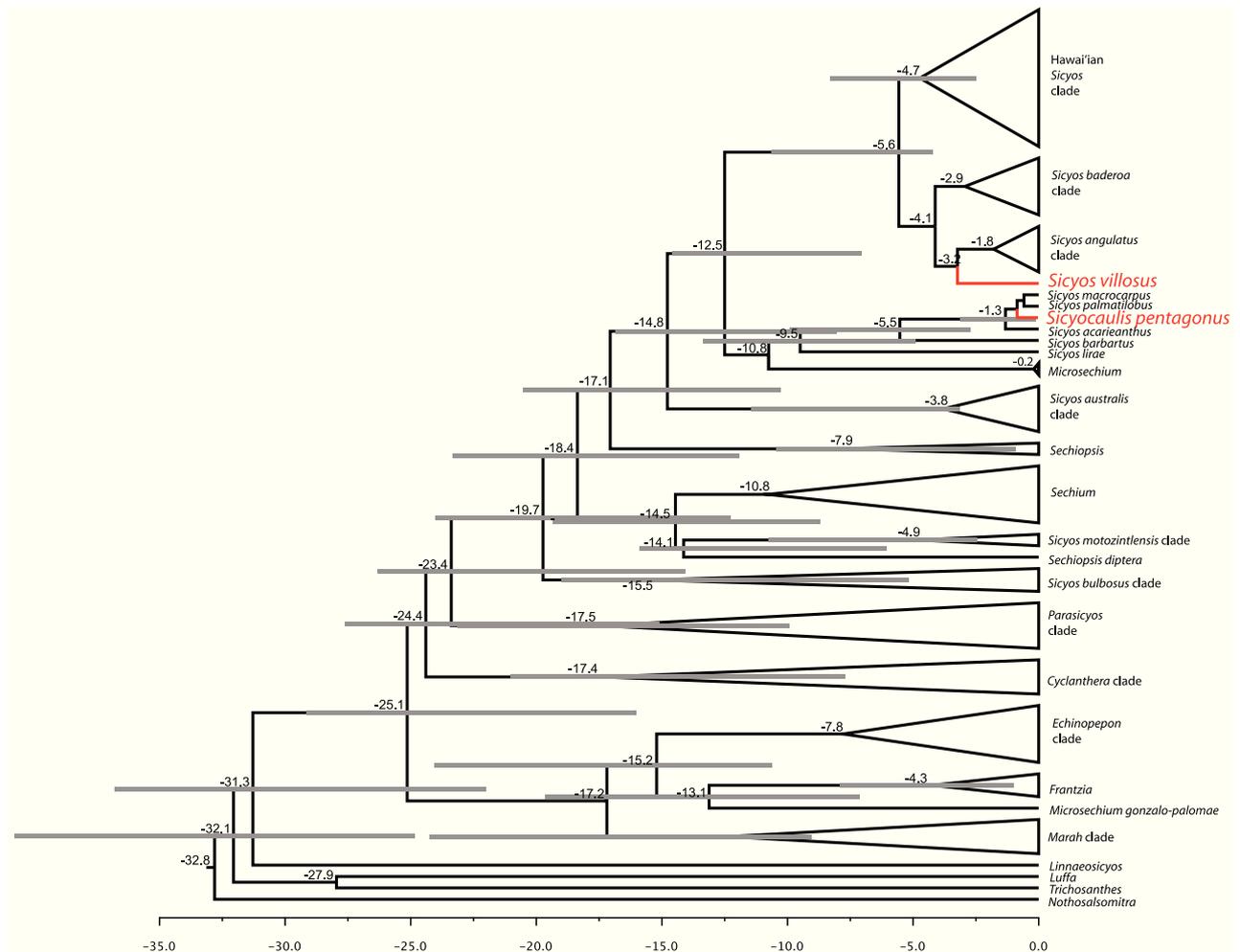
**Figure 1** The sole herbarium collection in existence of *Sicyos villosus*, collected by Darwin during his visit to Floreana in September 1835 and described as a new species by J. D. Hooker. The specimen is now in the Darwin herbarium, whose keeper, Professor J. Parker, granted permission to extract DNA from one of the seeds shown in the inset in the lower right (inset scale bar = 5 mm). Photograph by A. Tye.

species representing all these genera were sequenced for the chloroplast regions *rpl20-rps12* and *trnL/trnL-F*, and the entire nuclear ribosomal RNA intergenic spacer region (ITS1–5.8S–ITS2), following the methods described in Kocyan *et al.* (2007) and Schaefer *et al.* (2008). The genus *Sicyos* was represented with 41 of its *c.* 60 species. Trees were rooted on *Nothoalsomitra* (Schaefer *et al.*, 2009). Herbarium vouchers and GenBank accession numbers are given in the cited papers and in a phylogenetic study of *Sicyos* (P. Sebastian, H. Schaefer, and S. Renner, in preparation). Sequences were edited with SEQUENCHER (v.4.9; Gene Codes, Ann Arbor, MI, USA) and aligned by eye, using MACCLADE v.4.06 (Maddison & Maddison, 2000). The aligned plastid matrix comprised 1818 nucleotides, the aligned ITS matrix comprised 644 nucleotides. Analyses of the

separate plastid and nuclear data partitions produced congruent phylogenetic estimates, and the data were therefore concatenated. The complete alignment is available from P.S. Maximum likelihood (ML) analyses as well as ML bootstrapping relied on RAxML v.7.0.4 (Stamatakis *et al.*, 2008; available at: <http://phylobench.vital-it.ch/raxml-bb/>) and used the GTR + G model.

To obtain absolute ages for the divergences between the Galapagos Islands species and their mainland relatives, we used Bayesian time estimation with an uncorrelated-rates model as implemented in BEAST v.1.4.8 (Drummond & Rambaut, 2007). The alignment used for dating excluded all gapped positions and comprised 2113 nucleotides and 79 species. We again used the GTR + G model with four rate categories. Fossil calibration came from

the pollen *Hexacolpites echinatus* from the Oligocene of Cameroon (Salard-Cheboldaeff, 1978; Muller, 1985), which is the oldest known hexacolpate Sicyoeae-type pollen. The most conservative assignment of this pollen is to the split between *Limnaeosicyos* versus the remaining New World Sicyoeae (Schaefer *et al.*, 2009). The Oligocene epoch ranges from 33.9 to 23 Ma, and the stratum containing *Hexacolpites* has not been precisely dated; we used an age of  $28.5 \pm 6$  Ma because an analysis of Cucurbitaceae divergence times using first the upper then the lower boundary of the Oligocene found no significant difference (Schaefer *et al.*, 2009). We also constrained the root of the tree to  $37 \pm 3$  Ma based on Schaefer *et al.* (2009). Markov chain Monte Carlo runs extended for five million generations,



**Figure 2** Chronogram obtained under a relaxed clock model applied to 2113 aligned nucleotides (after exclusion of all gaps) from chloroplast and nuclear DNA sequences from 79 accessions of Sicyoeae (Cucurbitaceae). Darwin's *Sicyos villosus* is closest to species from North America and Mexico, while the Santa Cruz gourd, *Sicyocaulis pentagonus*, is closest to species from Peru and Ecuador; the two lineages also reached the archipelago at different times.

sampling every 1000th generation. Of the 5001 posterior trees, we excluded the first 1000 as burn-in. Convergence was checked using TRACER v.1.4.1 (Rambaut & Drummond, 2007). The estimated covariance parameter was 0.68, and the 95% highest posterior density (HPD) did not enclose 0, justifying the hypothesis of non-autocorrelated rate variation. Results under a strict clock model were similar to those obtained with the relaxed clock model.

A chronogram for 79 species of Sicyoeae–Cucurbitaceae, including 68% of the *c.* 60 species currently assigned to *Sicyos*, shows that both Galapagos cucurbits belong to this widespread genus, which has also diversified in the Hawaiian archipelago and in Australia and New Zealand (Fig. 2). Darwin's *Sicyos villosus* is closest to species from North America and Mexico, while the Santa Cruz gourd, *Sicyocaulis pentagonus*, is closest to species from Peru and Ecuador. The molecular clock suggests that the divergence from their respective closest relatives occurred 4 ( $\pm$  2) Ma in *Sicyos villosus*, and 1.4 ( $\pm$  1.2) Ma in *Sicyocaulis pentagonus*. Thus, the two species arrived on the Galapagos archipelago through non-anthropogenic long-distance dispersal from different continental source populations and at times that match the geological age of the islands: the Galapagos Islands are the product of hotspot activity 930 km west of the Ecuadorian coast and are at least 3–4 Myr old (Hickman & Lipps, 1985). Most plant species (up to 60%) appear to have arrived via birds, and the closest floristic ties are with Ecuador and Peru, followed by Central America and Mexico (Porter, 1976). It is also known that storm petrels migrate between Peru and the Galapagos (Tomkins, 1982) and that these and other seabirds nest in habitats where *Sicyos* occurs (Marks, 1992), suggesting that the spiny fruits (Fig. 1) may have been carried by birds.

Floreana, where Darwin collected *Sicyos villosus*, was settled in 1807 and continuously inhabited with only short breaks of a few years' abandonment during the 19th century. The settlers brought livestock, and by the time Darwin visited (in 1835) they owned approximately 2000 head of cattle (Steadman, 1986). Markham, who visited Floreana in 1880, found it 'in undisturbed possession of the so-called wild cattle ... donkeys, dogs, pigs, and other animals that had been left to run wild on the abandonment of the island by the former inhabitants' (Steadman, 1986, p. 62). A plausible explanation for the disap-

pearance of Darwin's gourd could be that it was grazed to extinction. And yet many Galapagos plants may be adapted to grazing: prior to the arrival of feral animals some 2–3 million giant tortoises (*Geochelone nigra*) lived on the archipelago and fed on its vegetation (Fowler de Neira, 1985; Coblentz & Baber, 1987). Tortoises, however, are inefficient grazers compared with goats and cattle, and the reach of the animals is different. There are many examples of Galapagos plants being apparently or obviously grazed to extinction or extreme rarity (A. Tye, personal communication, 18 January 2010). However, the speed of the disappearance of a plant described as abundant in 1835 is surprising.

Another plausible cause for the decline of *Sicyos villosus* (and possibly also *Sicyocaulis pentagonus*) could be Cucurbitaceae-specific viruses (e.g. cucumber mosaic virus, watermelon mosaic virus, zucchini yellow mosaic virus) introduced with cucurbit crops cultivated by the settlers. The dramatic decline of *Sicyos australis* in New Zealand, a species now almost entirely restricted to small offshore islands where no cucurbit crops are grown, has been attributed at least partly to these viruses (Delmiglio & Pearson, 2006). Similar inadvertent introduction of cucumber viruses could have occurred on the Galapagos Islands. Finally, it is possible that the extinction of Darwin's Galapagos gourd is only apparent and due to infrequent and uneven botanical collecting. In the course of this project, we contacted four botanists who have resided on the Galapagos Islands and made systematic collections there (Henning Adersen, Ole Hamann, Henk van der Werff and Alan Tye). All of them stressed that there are still some areas that have never been subject to a full botanical survey.

As illustrated by the sole specimen in existence of *Sicyos villosus*, Darwin's unique natural history collections continue to shed light on the origin and destruction of the Galapagos biota even 175 years after his visit. And although Darwin himself did not comment on the likely mode of transport of *Sicyos* seeds or fruits, he was acutely aware of the role of birds in plant dispersal (Briggs, 2009) and may well have collected the spiny seeds of *Sicyos villosus* (Fig. 1, inset) with this hypothesis in mind.

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## REFERENCES

- Briggs, J.C. (2009) Darwin's biogeography. *Journal of Biogeography*, **36**, 1011–1017.
- Coblentz, B.E. & Baber, D.W. (1987) Biology and control of feral pigs on Isla Santiago, Galapagos, Ecuador. *Journal of Applied Ecology*, **24**, 403–418.
- Delmiglio, C. & Pearson, M.N. (2006) Effects and incidence of cucumber mosaic virus, watermelon mosaic virus and zucchini yellow mosaic virus in New Zealand's only native cucurbit, *Sicyos australis*. *Australasian Plant Pathology*, **35**, 29–35.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214. doi:10.1186/1471-2148-7-214.
- Fowler de Neira, L.E. (1985) Diets of giant tortoises and feral burros on Volcan Alcedo, Galapagos. *Journal of Wildlife Management*, **49**, 165–169.
- Hickman, C.S. & Lipps, J.H. (1985) Geologic youth of Galapagos Islands confirmed by marine stratigraphy and paleontology. *Science*, **227**, 1578–1580.
- Kocyan, A., Zhang, L.-B., Schaefer, H. & Renner, S.S. (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553–577.
- Maddison, D.R. & Maddison, W.P. (2000) *MacClade*. Sinauer Associates, Sunderland, MA.
- Marks, J.S. (1992) Breeding biology of Tristram's storm-petrel on Laysan Island. *Wilson Bulletin*, **104**, 719–731.
- Muller, J. (1985) Significance of fossil pollen for angiosperm history. *Annals of the Missouri Botanical Garden*, **71**, 419–443.
- Porter, D.M. (1976) Geography and dispersal of Galapagos Islands plants. *Nature*, **264**, 745–746.
- Rambaut, A. & Drummond, A.J. (2007) *Tracer v. 1.4.1*. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Salard-Cheboldaeff, M. (1978) Sur la palynoflore Maestrichtienne et Tertiaire du bassin sédimentaire littoral du Cameroun. *Pollen et Spores*, **20**, 215–260.

- Schaefer, H., Kocyan, A. & Renner, S.S. (2008) *Linnaeosicyos* (Cucurbitaceae): a new genus for *Trichosanthes amara*, the Caribbean sister species of all Sicyoeae. *Systematic Botany*, **33**, 349–355.
- Schaefer, H., Heibl, C. & Renner, S.S. (2009) Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 843–851.
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology*, **57**, 758–771.
- Steadman, D.W. (1986) *Holocene vertebrate fossils from Isla Floreana, Galápagos*. Smithsonian Institution Press, Washington, DC.
- Tomkins, R.J. (1982) Migration of white-faced storm-petrels *Pelagodroma marina* in the South Pacific and the status of the Kermadec subspecies. *Emu*, **84**, 32–35.

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## Lessepsian fish migration: genetic bottlenecks and parasitological evidence

### ABSTRACT

As a rule, non-indigenous species (NIS) populations derived from biological invasion events represent a subset of the genetic diversity of the source population. In biological invasions, host–parasite interactions play an important role, and parasitological data for NIS populations can provide useful information such as their area of origin, mechanism of invasion and prospects of success in the new habitat. When both genetic and parasitological data are available, and they suggest the same scenario, the history of an invasion can be inferred with no discrepancy, but when data cannot be reconciled an alternative model should be considered. In this study a comparison of genetic and parasitological data for the Lessepsian migrant the bluespotted cornetfish, *Fistularia commersonii*, in the Mediterranean Sea presents the opportunity to evaluate the compatibility of information of this nature, and to propose possible

invasion scenarios consistent with evidence provided by both criteria.

**Keywords** Biological invasions, fish migration, *Fistularia commersonii*, genetics, Lessepsian migrations, Mediterranean Sea, parasitology.

### GENETIC AND PARASITOLOGICAL ASPECTS OF SPECIES INVASIONS

Genetic data can be useful for elucidating the history and phenomena involved in species invasions. In recent years, it has increasingly been emphasized that many natural populations that derived from a founder event and/or a bottleneck represent a subset of the genetic diversity of the source population, leading to the conclusion that the genetic diversity of the newly founded populations should be significantly lower than that of the source (Tsutsui *et al.*, 2000; Moum & Arnason, 2001; Sax *et al.*, 2005). Conversely, other types of evidence could also be used to retrace the route taken by invasive species. For instance, parasitological data might provide useful information about the area of origin of invaders, but might also shed light on some further aspects such as the mechanism of invasion (e.g. whether they arrived as adults or in the larval stages) or the prospects of success in the new habitat (e.g. potential infection with native and/or natural parasites) (Diamant, 1989; Populin & Mouillot, 2003; Galli *et al.*, 2007; Merella *et al.*, 2007; Pais *et al.*, 2007, 2008).

When both genetic and parasitological data are available, and suggest the same invasion scenario, the history of a particular invasion can be inferred with a certain degree of reliability. However, when these data on the same invasive species cannot be reconciled because they suggest a different route or history – assuming that both genetic and parasitological evidence are equally valid – an alternative model should be considered.

In this study a comparison of genetic and parasitological data for a Lessepsian migrant fish species presents the opportunity to suggest a multiple-approach method for a better understanding of the events that led to the invasion of the new range. In fact, the complementarity of such information greatly strengthens likely invasion scenarios suggested by both types of criteria.

### LESSEPSIAN FISH MIGRANTS AND GENETIC DIVERSITY

Since the opening of the Suez Canal in 1869, the Mediterranean Sea has been subject to an invasion of Indo-Pacific species from the Red Sea (Por, 1971). As far as fishes are concerned, it has recently been estimated that the so-called Lessepsian migrants include more than 60 species (Ben Rais Lasram *et al.*, 2008). Contrary to the evidence of a decrease in genetic diversity of invader species, analyses carried out to date on Lessepsian fish species reveal no significant reduction in genetic diversity, that is, conspecific populations from the Red Sea–Indo-Pacific region and the Mediterranean basin are genetically similar (Bucciarelli *et al.*, 2002; Hassan *et al.*, 2003; Hassan & Bonhomme, 2005). A likely explanation is that Lessepsian fish species invaded the Mediterranean with a conspicuous number of individuals, either as adults or larval stages, which reduced the loss of genetic diversity.

### THE CASE OF *FISTULARIA COMMERSONII*

The bluespotted cornetfish, *Fistularia commersonii* Rüppell (Osteichthyes: Fistulariidae), is an Indo-Pacific species whose presence was recorded for the first time on the Mediterranean coast of the Middle East (Golani, 2000). Later, its geographical distribution rapidly extended to the eastern basin (Corsini *et al.*, 2002), and more recently to the central and western Mediterranean (Pais *et al.*, 2007; Dulčić *et al.*, 2008). Knowledge of the specific dynamics of the Mediterranean invasion by *F. commersonii* is meagre. Golani *et al.* (2007), studying the partial sequence of the mitochondrial (mt) DNA control region (D-loop 1, primers in Lee *et al.*, 1995), suggest the occurrence of a genetic bottleneck in the Lessepsian migration of this species. These authors believe that the very low richness and diversity of the haplotypes found in the Mediterranean Sea (52 individuals, 2 haplotypes, haplotype diversity 0.009), compared with those from the natural range (49 individuals, 46 haplotypes, haplotype diversity 0.997), indicate that ‘...the Mediterranean populations of bluespotted cornetfish represent a single invasion event by as few as two females’ (Golani *et al.*, 2007, p. 544). The same authors attribute the rapid expansion of this fish in the Mediterranean Sea to larval dispersal favoured by water circulation.

## CHAPTER 4

RADIATION FOLLOWING LONG-DISTANCE DISPERSAL: THE  
CONTRIBUTIONS OF TIME, OPPORTUNITY, AND DIASPORE  
MORPHOLOGY IN *SICYOS* (CUCURBITACEAE)

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## Abstract

**Aim** To infer the most plausible explanations for the presence of 14 species of the Neotropical cucurbit genus *Sicyos* on Hawaii, two on the Galápagos Islands, two in Australia, and one in New Zealand.

**Location** Neotropics, the Hawaiian and Galápagos archipelagos, Australia and New Zealand.

**Methods** We tested long-problematic generic boundaries in the tribe Sicyoeae and reconstructed the history of *Sicyos* using plastid and nuclear DNA sequences from 87 species (many with multiple accessions) representing the group's generic and geographic diversity. Maximum likelihood and Bayesian approaches were used to infer relationships, divergence times, biogeographic history, and ancestral traits.

**Results** Thirteen smaller genera, including *Sechium*, are embedded in *Sicyos*, which when recircumscribed as a monophyletic group comprises 75 species. The 14 Hawaiian species of *Sicyos* descend from a single ancestor that arrived *c.* 3 million years ago (Ma), Galápagos was reached twice at *c.* 4.5 and 1 Ma, the species in Australia descend from a Neotropical ancestor (*c.* 2 Ma), and New Zealand was reached from Australia. Time since arrival thus does not correlate with *Sicyos* species numbers on the two archipelagos.

**Main conclusions** A plausible mechanism for the four trans-Pacific dispersal events is adherence to birds of the tiny hard fruit with retrorsely barbed spines found in those lineages that underwent long-distance migrations. The Hawaiian clade has lost these spines, resulting in a lower dispersal ability compared to the Galápagos and Australian lineages, favouring allopatric speciation in the diverse habitats of the archipelago.

## Introduction

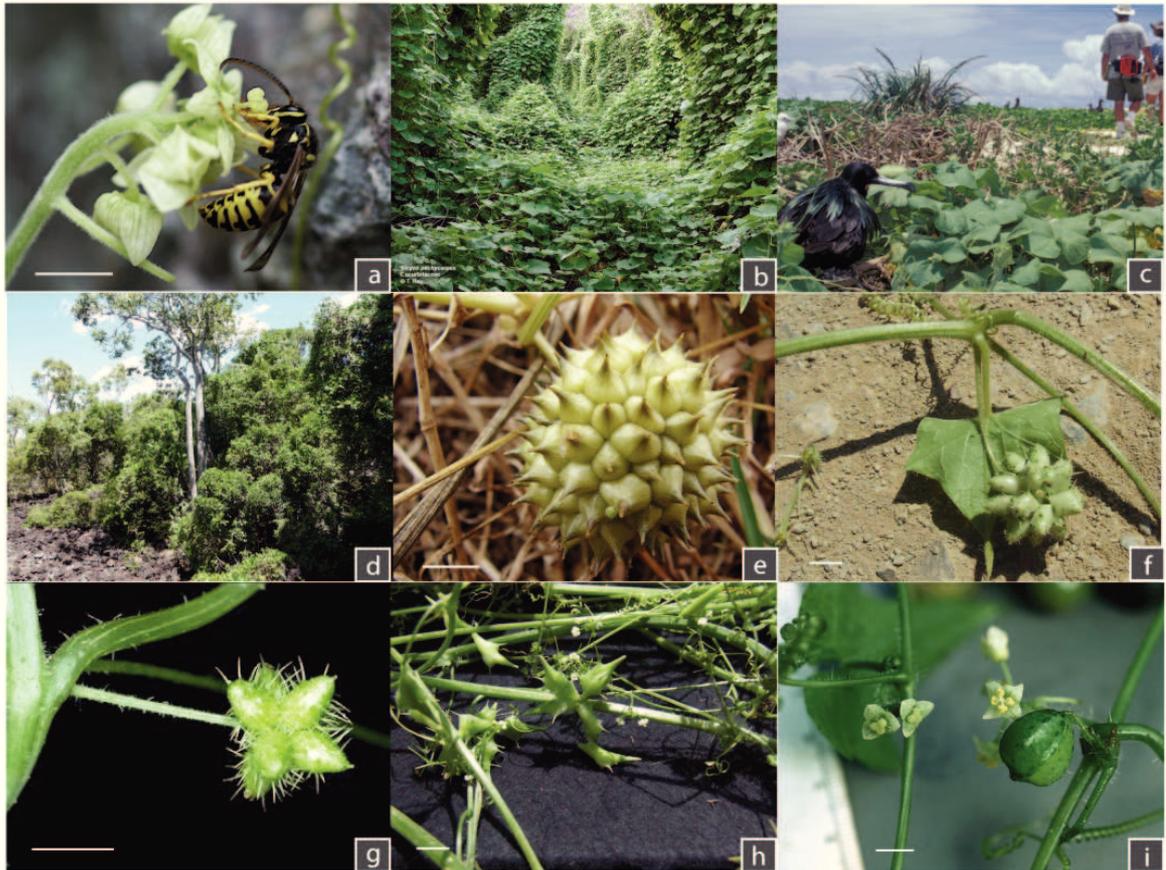
The geographic origin and speed of diversification of flowering plant clades occurring on the archipelagos of the Pacific Ocean have attracted much recent attention (e.g., Wright, 2000; Cronk *et al.*, 2005; Harbaugh & Baldwin, 2007; Clark *et al.*, 2008; Harbaugh *et al.*, 2009; Keppel *et al.*, 2009). Especially striking are radiations on the Hawaiian Islands, including the Lobelioideae with 126 species, *Cyrtandra* (Gesneriaceae) with 59, *Melicope/Platydesma* (Rutaceae) with 52, and eight more genus-level clades each with  $\geq 19$  species (Baldwin & Wagner, 2010). Most Hawaiian lineages are younger than five million years (Myr) (Price & Clague, 2002; Lindqvist & Albert, 2002; Clark *et al.*, 2009; Havran *et al.*, 2009; Willyard *et al.*, 2011), implying extreme diversification rates. The Galápagos, by contrast, have produced no plant radiations with more than a few species, among them the daisy genus *Scalesia* with 15 species that may go back to a common ancestor living 1.9 - 6.2 million years ago (Ma)

(Schilling *et al.*, 1994), and *Varronia* with four species dated to 1.12 - 4.5 Ma (Weeks *et al.*, 2010). The difference in the number and size of plant radiations on the two archipelagos could reflect time available for diversification, ecological opportunity, and different clades' propensity to form isolated populations prone to interruption of gene flow. Other possible explanations include different extinction effects (e.g., due to different palaeoclimatic history) or taxonomic bias (different species concepts applied on different archipelagos; Carine & Schaefer 2010; Schaefer *et al.*, 2011).

Clades occurring on both Pacific archipelagos in principal should allow disentangling the contribution of time and ecological opportunity from clade-immanent propensity to form new species. The propensity to form new species may correlate with dispersal ability, mating system, ease of hybridization, and speed of karyotype rearrangements affecting a group's ability to form viable polyploids. The indigenous floras of the Hawaiian and Galápagos archipelagos share only 24 plant genera, and 13 of these genera have evolved endemic species on both archipelagos (Table 1). Among them is *Sicyos*, the focal clade of this study. *Sicyos* is a genus in the Cucurbitaceae that has 14 endemic species on the Hawaiian Islands (Wagner & Shannon, 1999), two species on the Galápagos Islands (Sebastian *et al.*, 2010a), two in Australia, one in New Zealand, and between 41 and 56 species in the Americas, depending on taxonomic concept applied; several small genera have been included in *Sicyos* or segregated from it based mostly on fruit characters (*Materials and Methods*). Species of *Sicyos* are climbing or trailing annual or perennial vines that often occur in disturbed habitats. All *Sicyos* species are monoecious, with male and female flowers on each individual; the flowers are diurnal and depend on wasps and short-tongued bees for pollination (LaBerge & Hurd, 1965; Fig. 1a) since automatic selfing is precluded by their unisexuality. The clade, including the segregate genera, is especially diverse in Mexico, where several new species have been discovered in the recent past (Lira, 1994; Lira & Rodríguez-Arevalo, 1999; Rodríguez-Arevalo & Lira, 2001; Rodríguez-Arevalo, 2003; Rodríguez-Arevalo *et al.*, 2004, 2005). *Sicyos* is the name-giving taxon of the Sicyoeae, a tribe with 265 species in perhaps a dozen genera (Schaefer & Renner, 2011a, b). Family-wide molecular phylogenies relying on plastid and recently also nuclear data suggest that Sicyoeae are monophyletic (Kocyan *et al.*, 2007; Schaefer *et al.*, 2009; Schaefer & Renner, 2011b). However, none sampled more than a few species of *Sicyos*.

Here we use *Sicyos* to study whether time since arrival, ecological opportunity, or lineage-specific factors more plausibly explain the different species numbers produced in regions reached by long-distance dispersal, namely Hawaii (14 species), Galápagos (2),

Australia (2) and New Zealand (1). A single medium-sized clade having produced species in all these areas provides a rare opportunity to disentangle the relative effects of age, traits and ecological opportunity on diversification.



**Fig. 1.** Habitats, fruits or flowers of: (a) *S. angulatus* visited by *Vespula germanica* (USA), (b) *S. pachycarpus* (Hawaii), (c) *S. maximowiczii* growing in a colony of Great Frigatebird (*Fregata minor*; Hawaii), (d) *S. sp. nov. 'undara'* (Australia), (e) *S. pachycarpus* (Hawaii), (f) *S. weberbaueri* (Peru), (g) *S. australis* (Australia), (h) *S. acarieanthus* (Peru), (i) *Microsechium ruderale* (Guatemala). Scale bar = 1cm. Photographs by: H. Schaefer (a), T. Rau (b; Carr, 2006), Forest & Kim Starr (c & e), P. Sebastian (d), M. Weigend (f, h), A. Lyne (g; APII), and M. Nee (i).

**Table 1.** Shared native angiosperm genera of the Hawaiian and Galápagos Islands. In bold, genera with endemic species on both archipelagos (data from: Wiggins & Porter, 1971; Wagner *et al.*, 1990; Carr, 2006; Bungartz *et al.*, 2009).

Genus	Family	Hawaii		Galápagos	
		Endemic	Native	Endemic	Native
<b><i>Abutilon</i></b>	<b>Malvaceae</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>0</b>
<i>Acacia</i>	Mimosaceae	2	0	0	3
<b><i>Amaranthus</i></b>	<b>Amaranthaceae</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>1</b>
<i>Cordia/Varronia</i>	Boraginaceae	0	1	4	2
<b><i>Cuscuta</i></b>	<b>Cuscutaceae</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>0</b>
<i>Dodonaea</i>	Sapindaceae	0	1	1	0
<b><i>Chamaesyce</i></b>	<b>Euphorbiaceae</b>	<b>15</b>	<b>0</b>	<b>8</b>	<b>0</b>
<b><i>Gossypium</i></b>	<b>Malvaceae</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>0</b>
<i>Heliotropium</i>	Boraginaceae	0	2	1	3
<b><i>Ipomaea</i></b>	<b>Convolvulaceae</b>	<b>1</b>	<b>4</b>	<b>2-3</b>	<b>3</b>
<i>Lobelia</i>	Campanulaceae	13	0	0	1
<i>Lycium</i>	Solanaceae	0	1	1	0
<b><i>Peperomia</i></b>	<b>Piperaceae</b>	<b>23</b>	<b>2</b>	<b>4</b>	<b>2</b>
<i>Phyllanthus</i>	Euphorbiaceae	1	0	0	1
<i>Phytolacca</i>	Phytolaccaceae	1	0	0	1
<i>Pilea</i>	Urticaceae	0	1	1	1
<b><i>Pisonia</i></b>	<b>Nyctaginaceae</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>0</b>
<b><i>Plantago</i></b>	<b>Plantaginaceae</b>	<b>3</b>	<b>0</b>	<b>1</b>	<b>0</b>
<i>Plumbago</i>	Plumbaginaceae	0	1	0	2
<b><i>Portulaca</i></b>	<b>Portulacaceae</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>0</b>
<b><i>Psychotria</i></b>	<b>Rubiaceae</b>	<b>11</b>	<b>0</b>	<b>2</b>	<b>0</b>
<i>Sesuvium</i>	Aizoaceae	0	1	1	1
<b><i>Sicyos</i></b>	<b>Cucurbitaceae</b>	<b>14</b>	<b>0</b>	<b>2</b>	<b>0</b>
<b><i>Solanum</i></b>	<b>Solanaceae</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>1</b>
<i>Waltheria</i>	Sterculiaceae	0	1	0	1

## Materials and Methods

### Taxon sampling, DNA sequencing, alignment and phylogenetic analysis

We used 112 accessions representing 87 species of Sicyoae, including the type species of all relevant generic names so as to be able to decide the taxonomic fate of the previously segregated genera *Anomalosicyos* Gentry (7 spp., Central to South America), *Cladocarpa* (St. John) St. John (20 spp./names, Hawaii), *Costarica* L.D. Gómez (1 sp., Costa Rica), *Microsechium* Naudin (2 - 4 spp., Mexico, Guatemala), *Parasicyos* Dieterle (2 spp., Guatemala), *Pterosicyos* Brandegees (1 sp., Mexico and Guatemala), *Sarx* St. John (2 spp./names, Hawaii), *Sechiopsis* Naudin (5 spp., Mexico and Guatemala), *Sechium* P. Browne (5 spp. Mexico), *Sicyocarya* (A. Gray) St. John (25 spp./names, Hawaii), *Sicyocaulis* Wiggins

(1 sp., Galápagos Islands), *Sicyosperma* Gray (1 sp., Mexico and Arizona) and *Skottsbergiliana* St. John (2 spp./names, Hawaii). No previous taxonomic fusions or segregations were based on molecular data. We were able to sample all species known from the Galápagos Islands, Australia, and New Zealand, and 13 of the 14 known from Hawaii. The missing Hawaiian species, *S. semitonsus*, is close to *S. herbstii*, *S. hispidus*, and *S. maximowiczii*, judging from the shared hairy fruit protuberances (Telford, 1990) but may actually be a hybrid (Starr & Martz, 1999). The type specimen of *Costarica hamata* was unavailable for sequencing, and we instead used material collected at the type locality on the slopes of the Irazú volcano in Costa Rica. Table S1 lists all included species with their authors, geographic origin of the sequenced sample, voucher deposition, and GenBank accession numbers. A total of 420 chloroplast and 98 nuclear sequences were newly generated for this study and have been submitted to GenBank (acc. no. JN560179 - JN560696).

Genomic DNA was isolated from herbarium specimens or from silica-dried leaves, using the NucleoSpin plant kit (Machery-Nagel, Düren, Germany). Polymerase chain reaction (PCR) protocols and primers were the same as in Sebastian *et al.* (2010b). The plastid DNA regions sequenced were the *trnL* intron, the adjacent *trnL* - *trnF* intergenic spacer, the *rpl20* - *rps12* intergenic spacer, the *trnS-trnG* intergenic spacer, the *psbA* - *trnH* intergenic spacer and the *rbcL* gene; the nuclear region sequenced was the complete internal transcribed spacer (ITS) region of the ribosomal DNA. For amplification of the *psbA* - *trnH* intergenic spacer, we used the primers listed in Volz & Renner (2009). The PCR products were purified with the PCR Wizard clean-up kit (Promega GmbH, Mannheim, Germany) or ExoSap (Fermentas, St. Leon-Rot, Germany). Cycle sequencing was performed with the BigDye Terminator cycle sequencing kit on an ABI Prism 3100 Avant automated sequencer (Applied Biosystems, Foster City, California, USA). Sequencing primers were the same as those used for DNA amplification. The ITS region yielded single bands and unambiguous base calls, and we therefore refrained from cloning. Sequence assembly of forward and reverse strands was carried out with Sequencher vs. 4.7 (Gene Codes, Ann Arbor, Michigan, USA), and aligned by eye using MacClade version 4.08 (Maddison & Maddison, 2003).

The aligned plastid DNA matrix comprised 4527 nucleotides and the aligned ITS matrix 872 nucleotides. In eight cases, we combined plastid and nuclear sequences coming from different samples (Table S1). Maximum likelihood (ML) analyses and ML bootstrap searches (using 500 replicates) were carried out using RAxML version 7.2.8 (Stamatakis, 2006). RAxML searches relied on the GTR +  $\Gamma$  model, with model parameters estimated over the duration of specified runs. Analyses of the separate plastid and nuclear datasets with one

exception yielded congruent tree topologies, with differences restricted to tip nodes with low statistical support (bootstrap < 75%); the sole difference concerned *Microsechium gonzalopalmiae*. This species was therefore excluded from our further analyses.

### **Molecular clock analyses and diversification rates**

To obtain absolute age ranges for the nodes of biogeographic interest, we used Bayesian time estimation and a relaxed clock uncorrelated-rates model as implemented in BEAST version 1.6.1 (Drummond & Rambaut, 2007). Species with nearly identical sequences were excluded from the dating analysis to reduce stochastic error and rate heterogeneity, yielding an alignment of 81 species, including the early-diverging Sicyoeae *Luffa aegyptiaca*, *Nothoalsomitra suberosa*, and *Trichosanthes ovigera* for rooting purposes (Schaefer & Renner, 2011b). The pollen *Hexacolpites echinatus* from the Oligocene (33.9 to 23 Ma) of Cameroon (Salard-Chebouldaëff, 1978) is the oldest known hexacolpate echinate Sicyoeae-type pollen and was used as a calibration point. The most conservative assignment of this pollen is to the split between *Linnaeosicyos* with 4 - colporate reticulate pollen (Schaefer *et al.*, 2008a) and the remaining New World Sicyoeae with 4 - 16 colpate/colporate and mostly echinate pollen (Schaefer *et al.*, 2009; Sebastian *et al.*, 2010a). To cover the uncertainty in the pollen age, we applied a normally distributed prior probability distribution of  $28.5 \pm 6$  Ma to this node. The root of the Sicyoeae was constrained to  $37 \pm 3$  Ma (again with a normal prior distribution) based on the age found for this node in the family-wide analysis by Schaefer *et al.* (2009). All BEAST runs used a Yule tree prior and the GTR +  $\Gamma$  model with six rate categories; Monte Carlo Markov chains (MCMC) were run for 20 million generations, sampling every 1000<sup>th</sup> generation. Mixing of the chains and convergence were checked using Tracer version 1.5 (Rambaut & Drummond, 2007); of the 20,001 posterior trees, the first 5,000 were discarded as burn-in based on inspection of the Tracer files. Final trees were edited in FigTree version 1.3.1 (Rambaut, 2006).

We modeled diversification as a time-homogeneous birth/death process, with a net diversification rate  $r$  and relative extinction rates  $\kappa = 0$  or  $\kappa = 0.9$  (Magallón & Sanderson, 2001). Rates were calculated using the rate.estimate algorithm implemented in the R package GEIGER version 1.3.1 (Harmon *et al.*, 2008).

### **Ancestral area reconstruction**

To infer the geographical unfolding of Sicyoeae, we applied Bayesian MCMC searches in BEAST version 1.6.1 (Drummond & Rambaut, 2007). Analyses incorporated a continuous-time Markov chain (CTMC) phylogeographic model with stochastic search variable selection

(Lemey *et al.*, 2009). The advantage of this method is that it incorporates branch length information as well as uncertainty in the tree topology. Prior distributions for migration rates were defined by a GAMMA prior (shape = scale = 1.0) for the relative rate parameter and an exponential prior (mean = 1.0) on the geosite model parameter as recommended by Lemey *et al.* (2009). The CTMC phylogeographic model assumes that ancestral ranges are limited to single regions, making it particularly relevant for clades in which dispersal plays a larger role than disjunctions. The geographic regions coded were: (i) North American plate (ii) Caribbean plate, (iii) South American plate, (iv) Hawaii, (v) Galápagos, and (vi) Australia/New Zealand.

### **Evolution of habitat occupation and fruit armature**

To assess whether habitat diversity in a region is correlated with diversification (by promoting speciation), we categorized habitats into (i) shrublands and coastal vegetation, (ii) dry lowland forest, (iii) tropical deciduous forest, (iv) tropical evergreen forest, (v) tropical wet montane or cloud forest, (vi) *Pinus* - *Quercus* forest, and (vii) dry montane forest, and coded each species for its preferred habitat(s). Data on habitat preferences came from taxonomic and floristic treatments (Macbride, 1960; Wiggins & Porter, 1971; Wagner *et al.*, 1990; Jeffrey & Trujillo, 1992) and from specimen labels. Preferences were plotted on the 86-species ML phylogeny (*Results*).

Fruit and seed morphology in the Sicyoeae is exceptionally variable, and traits such as fleshy or hard fruits, with smooth surfaces or surfaces bearing barbed or hooked spines, likely influence dispersal. To infer ancestral states of fruit armature in *Sicyos*, we used maximum likelihood as implemented in Mesquite version 2.74 (Maddison & Maddison, 2009), employing the Markov k-state one-parameter model, which is a generalized Jukes-Cantor model (Lewis, 2001). Transition parameters were estimated on the 86-species ML phylogram. The coded fruit character states were: (i) armed, (ii) unarmed, (iii) winged, and (iv) within-species variation in fruit armature.

## Results

### Phylogenetic relationships of the Sicyoeae

The maximum likelihood phylogeny for the Sicyoeae (Fig. 2) shows that all species of *Anomalosicyos*, *Cladocarpa*, *Costarica*, *Microsechium* (as to its type species, *M. ruderale*), *Parasicyos*, *Pterosicyos*, *Sarx*, *Sechiopsis*, *Sechium*, *Sicyocarya*, *Sicyocaulis*, *Skottsbergiliana*, and *Sicyosperma* are embedded among species of *Sicyos*, a clade that itself has 100% bootstrap support. In addition, all the segregate genera that had more than one species (*Anomalosicyos*, *Microsechium*, *Parasicyos*, *Sechiopsis*, *Sechium*) turn out to be polyphyletic, and *Frantzia*, which traditionally was seen as close to *Sechium*, instead constitutes a genetically distant lineage (Fig. 2). From now on, we focus on the monophyletic genus *Sicyos* as circumscribed in Fig. 2, that is, including all the former segregates. The Hawaiian species of *Sicyos* descend from a common ancestor, and this is also supported by a six base pair deletion in their *trnL* intron. The single New Zealand species groups with the two Australian species. By contrast, the two species on the Galápagos archipelago result from independent dispersals to the islands (Fig. 2).

The Mexican species *Microsechium gonzalo-palomae*, which is the only species placed differently with plastid and nuclear (ITS) sequences (*Materials and Methods*), based on its plastid sequences clusters with the *Frantzia* clade, but based on its nuclear sequences belongs in *Sicyos*. Two ITS sequences from duplicates of one of the only two existing herbarium collections of this species showed ten nucleotide differences but nevertheless clustered together (Fig. 2), suggesting multiple coexisting ITS copies, such as would be expected following hybridization. Investigating the nature of this species or hybrid population will require fieldwork to collect more material.

### Divergence times, direction of dispersal, and diversification rates

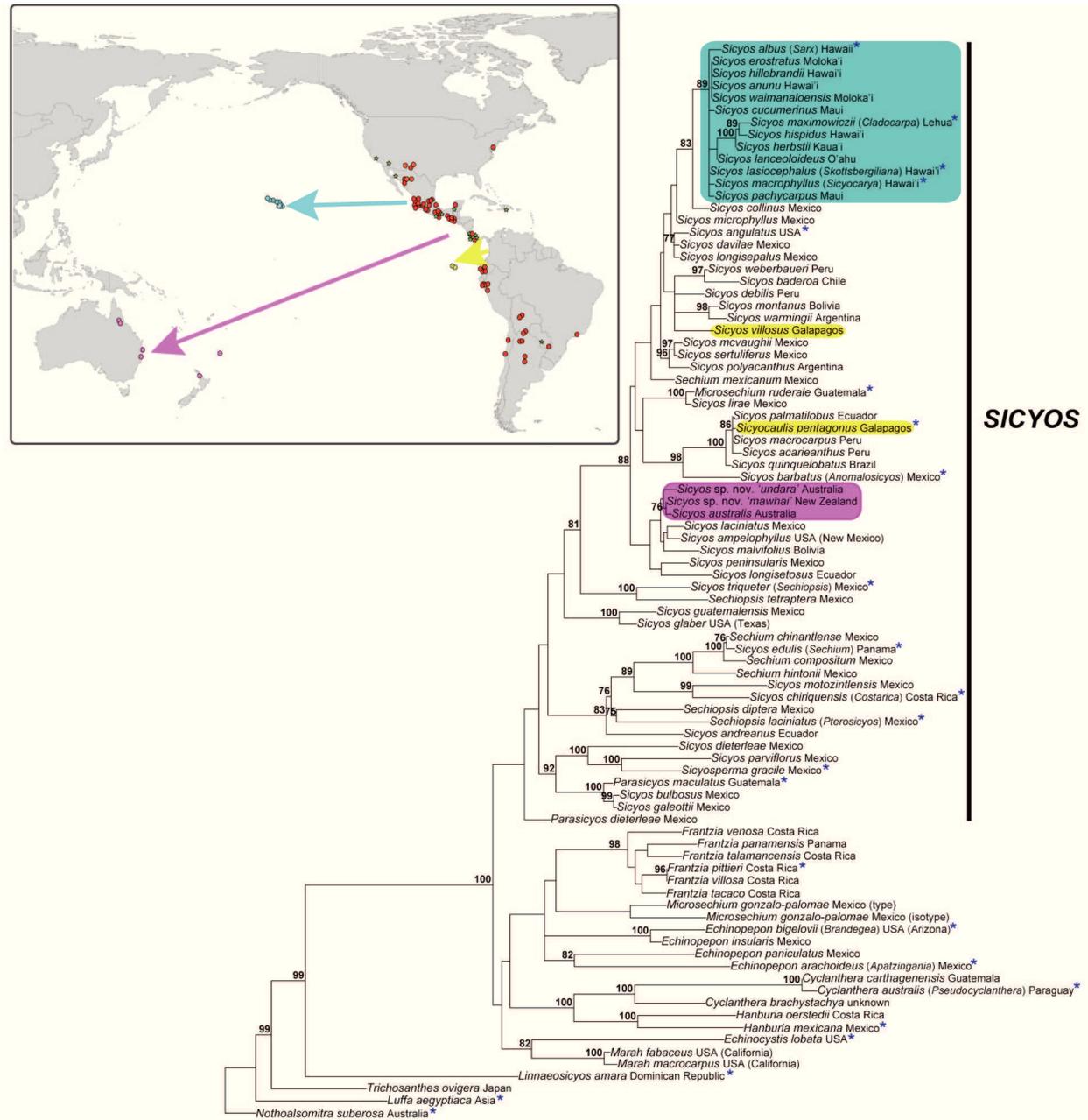
*Sicyos* originated in North America (Fig. S1; likely Mexico (inset Fig. 2)) during the early Miocene, 26.7 - 18.1 Ma (Figs. 3). The common ancestor of the Hawaiian radiation is inferred to have diverged from a North American (Mexican) lineage 5.5 - 1.9 Ma and to have given rise to the extant Hawaiian species around 4.1 - 1.3 Ma (Figs. 3 and S1). The Galápagos species *S. villosus* is part of a clade of species occurring in Ecuador, Peru, Chile, Bolivia, Argentina, and Southern Brazil from which it diverged about 6.4 - 2.8 Ma. The other Galápagos species, *Sicyocaulis pentagonus*, is nested among species from Ecuador, Peru, and Brazil from which it diverged 1.5 - 0.08 Ma so the ancestral areas of both Galápagos species likely were in South America, possibly in adjacent mainland Ecuador (Figs. 3 and S1). The

Australia/New Zealand clade is sister to species from the south-western United States, Mexico, and Bolivia, and it diverged from a North American ancestor 5.6 - 1.7 Ma. The Australian *S. sp. nov. 'undara'* diverged 3.6 - 0.5 Ma and its close relatives, *S. australis* and *S. sp. nov. 'mawhai'*, *c.* 1.6 - 0.1 Ma. The two new species differ from *S. australis* in fruit morphology, the number of flowers per inflorescence, flower size, and peduncle length and will be formally described elsewhere (Telford *et al.*, in review).

The net diversification rate ( $r$ ) of the *Sicyos* crown group in the Hawaiian Islands is  $r = 0.47 - 1.45$  species per Myr ( $\text{Myr}^{-1}$ ) assuming no extinction ( $\kappa = 0$ ) and  $r = 0.18 - 0.58$  species  $\text{Myr}^{-1}$  if extinction rates are high ( $\kappa = 0.9$ ; Table 2, which also summarizes the characteristics of the four trans-Pacific disjunctions). In the Australian/New Zealand clade, the diversification rate is  $r = 0.11 - 0.81$  per Myr ( $\kappa = 0$ ) or  $r = 0.03 - 0.24$  per Myr ( $\kappa = 0.9$ ), and of course is zero for the two single-species Galápagos lineages.

**Table 2.** Comparison of the characteristics of the four *Sicyos* disjunctions.

Characteristic	Hawaii	Australia / New Zealand	Galápagos 1 ( <i>S. villosus</i> )	Galápagos 2 ( <i>S. pentagonus</i> )
Number of extant species	14	3	1	1
Distance from likely region of origin [km]	3,800	6,000	930	930
Relative habitat diversity	high	high	low	low
Dispersal ability (diaspore morphology)	low	high	high	high
Stem age [Ma]	5.5 - 1.9	5.6 - 1.7	6.4-2.8	1.5-0.08
Crown age [Ma]	4.1 - 1.3	3.6 - 0.5	NA	NA
Diversification rate ( $\kappa=0$ ) [species $\text{Myr}^{-1}$ ]	0.47 - 1.45	0.11 - 0.81	0	0
Diversification rate ( $\kappa=0.9$ ) [species $\text{Myr}^{-1}$ ]	0.18 - 0.58	0.03 - 0.24	0	0



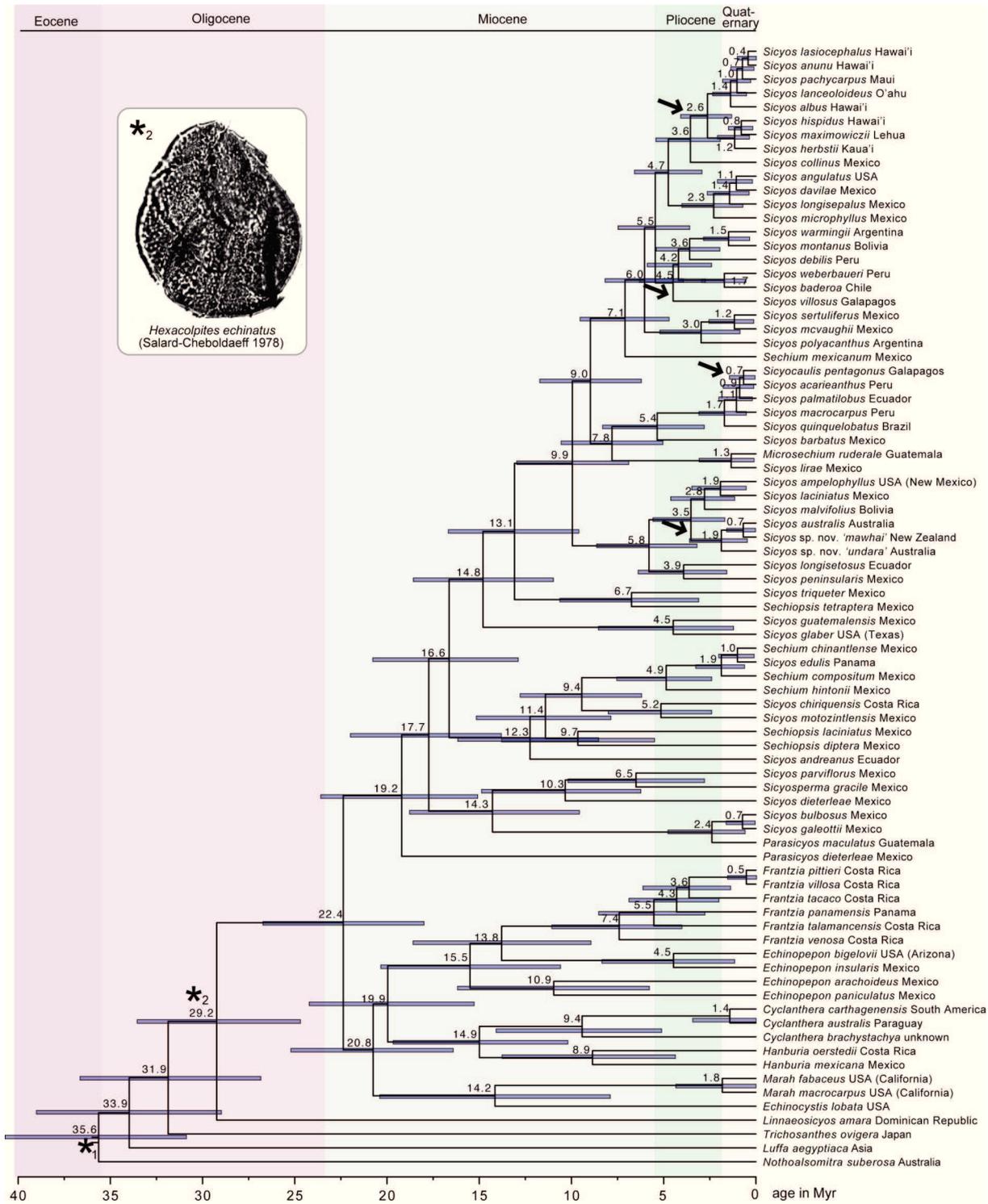
**Fig. 2.** Maximum likelihood phylogram for 87 species of Sicyoeae based on 5399 aligned nucleotides of plastid and nuclear sequences analysed under a GTR +  $\Gamma$  model. The tree is rooted on *Nothoalsomitra*. Values at nodes give likelihood bootstrap support of at least 75% based on 500 replicates. Boxes around clades and arrows in the inset mark the three long-distance dispersals to: Hawaii (blue), Galápagos (yellow), and Australia/New Zealand (pink). Stars indicate type species of currently or formerly accepted genera. Inset: Geographic origin of the sequenced plant material. Circles: *Sicyos*, stars: other Sicyoeae.

**Habitat diversity and fruit morphology**

Habitat preferences in *Sicyos* are extremely variable even within very young clades (Fig. S2). Some of the natural habitats are shown in Fig. 1. We did not code disturbed versus undisturbed habitats, however, *Sicyos* species often grow in disturbed sites. Some of the widespread species, such as *S. angulatus* and *S. polyacanthus*, occur in a wide range of habitats in their native range and are locally invasive in the Old World. Most Hawaiian species are found in coastal areas, arid or moist shrub lands, or openings in rain forest up to 2000 metres elevation. *Sicyocaulis pentagonus* on the Galápagos is known only from a few collections in moist forests of the *Scalesia* zone of the higher islands (between 130-200 and 400-550 m). The other Galápagos endemic, *Sicyos villosus*, is known from a single collection made by Charles Darwin on Floreana, which lacks habitat details. Habitats on Floreana, which has a maximum elevation of 640 m a.s.l., could have been coastal vegetation, arid shrublands, and/or moist *Scalesia* forest.

One of the two Australian species, *S. australis*, is widespread in eastern Australia, occurring at disturbed sites in deciduous vine thickets, eucalypt forest, and in montane and near-coastal habitats from northern Queensland to Tasmania; it has also been collected on New Zealand's North Island, Lord Howe and Norfolk Islands. The second Australian species, *S. sp. nov. 'undara'*, is only known from the Undara Volcanic National Park in Queensland, where it grows in clay loam in boulder gullies of collapsed lava tubes. The New Zealand endemic, *S. sp. nov. 'mawhai'*, is restricted to a small region on the North Island, adjacent islands, and the Kermadec Islands, where it occurs in shrubberies or forest margins of near-coastal sites.

Ancestral state reconstruction suggests that glabrous fruits and fruits armed with spines or hooks evolved several times (Fig. 1 for colour photos of armed or unarmed fruits from Hawaii, Australia, and the continental mainland; Fig. S3 for fruit trait ancestral state reconstruction). Both Galápagos species as well as the Australian and New Zealand species have spiny fruits and are derived from relatives with such fruits (Figs. 1g and S3). By contrast, the Hawaiian clade lost the spines and instead has smooth fruits (Fig. 1e) or fruits with stubby, hairy protuberances (*S. maximowiczii*, *S. hispidus*, *S. herbstii* and *S. semitonsus*).



**Fig. 3.** Chronogram for *Sicyos* and related Sicyoeae obtained under a Bayesian relaxed clock with log-normally distributed rates, applied to the dataset used for Fig. 2 but excluding five Hawaiian species with almost identical sequences. Bars at nodes indicate the 95% confidence intervals around the estimated times. Numbers above branches indicate the node age, stars the calibration nodes, and arrows the four long-distance dispersals discussed in the text.

## Discussion

Here we use a medium-sized clade (75 species in its new monophyletic circumscription) to study whether ecological opportunity or time available for speciation more plausibly explain strikingly different species numbers in four regions reached by long-distance dispersal. Our dense species sampling permitted identifying the geographic origin of the disjunct species on Hawaii, the Galápagos, Australia, and New Zealand. The distances from North America to Hawaii (3,800 km) and from the South American mainland (Ecuador) to Galápagos (930 km) should favour plant arrival on the Galápagos, but hardly make it so frequent as to retard speciation. Indeed, based on our biogeographic reconstruction, Hawaii, Australia, and New Zealand each were reached a single time, while Galápagos was reached twice. We estimate that the genus *Sicyos* is about 19 Myr old, the Hawaiian radiation 3 Myr, the Australian species 2 Myr, the New Zealand species 0.7 Myr, and the Galápagos species 4.5 and 1 Ma (for error margins see Table 2 and Fig. 3). Time *per se* therefore cannot explain the different species numbers in the four areas reached by long-distance dispersal. Instead, the species build-up on Hawaii has exceeded that in the other regions, although the Hawaiian diversification rate of *Sicyos* is not exceptional compared to other plant radiations (Valente *et al.* 2010). The Hawaiian *Sicyos* species are clearly distinct from each other (Wagner & Shannon, 1999), but their genetic divergence is low (Fig. 2), a combination also found in other Hawaiian radiations (Baldwin & Robichaux, 1995; Lindqvist *et al.*, 2003).

Since time for speciation can be discounted as an obvious explanation for the numbers of species in the four areas reached following long-distance dispersal, it might be extrinsic factors that make Hawaii especially conducive to *Sicyos* speciation. The Hawaiian archipelago harbours many types of plant formations (Mueller-Dombois & Fosberg, 1998), and species of *Sicyos* occupy most of them (Fig. S2). Especially important for *Sicyos* diversification may be the extreme breadth of rainfall regimes on Hawaii. High islands, such as Kaua'i and Hawai'i, on their windward slopes receive as much as 12 m annual precipitation, whereas their leeward slopes like some of the low islands during the warm season also experience droughts with as little as 250 mm of annual precipitation. The Hawaiian soils derived from volcanism have accordingly experienced very different weathering (Cuddihy, 1989).

The Galápagos archipelago, by comparison, has fewer climate and vegetation types. Aridification there set in at around 3 Ma, while before conditions were warmer and moister (Wara *et al.*, 2005; Federov *et al.*, 2006) and potentially supported rain forest vegetation. Today, its climate is characterized by highly variable convective rainfall during the hot season

and a prolonged cool season (June to December), with only little orographic rainfall in the higher windward sides of the islands and frequent droughts at lower elevations with arid-adapted vegetation types (Mueller-Dombois & Fosberg, 1998). The overall more arid climate on Galápagos compared to Hawaii, combined with lower number of different habitats on smaller islands, may explain the larger and more numerous plant radiations on the latter archipelago. Among the 13 angiosperm genera with endemic species in both archipelagos (Table 1) the Hawaiian ones tend to have more endemic species than the Galápagos genera, and radiations of more than four species also are concentrated on Hawaii.

An even broader range of climatic and edaphic conditions in the Australian region than in Hawaii, however, did not result in a similarly high number of *Sicyos* species, although further collecting and sequencing efforts in that region may well reveal additional species. The limited diversification of *Sicyos* in Australia resembles the situation in the remaining Cucurbitaceae lineages on that continent, which arrived over the past 35 - 1 Myr via minimally 20 dispersal events from the Malesian region (Schaefer *et al.*, 2008b, 2009). The largest cucurbit radiations in Australia are *Austrobryonia*, which over about eight million years evolved into four species in the arid and semiarid regions of the continent (Schaefer *et al.*, 2008b), and *Cucumis* with two radiations in tropical savanna habitats (Sebastian *et al.*, 2010b; Telford *et al.*, 2011). The reasons for limited cucurbit diversification in Australia remain unclear. In New Zealand, the family had no endemic species before the discovery of *S.* sp. nov. “*mawhai*”. Cucurbitaceae in general are successful transoceanic dispersers (Schaefer *et al.*, 2009; Schaefer & Renner, 2010; Duchon & Renner, 2010), nor is *Sicyos* the only angiosperm genus disjunctly distributed between the Americas and the Australian/New Zealand region. Californian species of *Lepidium* (Brassicaceae) are phylogenetically closest to Australian/New Zealand species, which has been explained by transoceanic dispersals (Mummenhoff *et al.*, 2004). The distance of 2153 km between Australia and New Zealand, perhaps covered by the progenitor of *S.* sp. nov. “*mawhai*” has been overcome by many other clades that are disjunctly distributed in Australia and New Zealand (Pole, 1994).

Seabirds are known to act as dispersal agents across the Pacific Ocean (Falla, 1960; Carlquist, 1967). Storm petrels, shearwaters, and frigate birds nest on the ground or in burrows in coastal vegetation or more inland, and they may contact fruiting *Sicyos* plants growing in their colonies (Marks & Leasure, 1992; Starr & Martz, 1999; our Fig. 1c shows a Great Frigate Bird nesting among *S. maximowiczii* on Laysan Island, Hawaii). Some petrel populations migrate between the Central and South American mainland, the Galápagos, and Hawaii, and one race of White-faced Storm-Petrels migrates across the Pacific between New

Zealand and the Humboldt Current (off the coast of Peru), then west past the Galápagos Islands (Tomkins, 1982). Species of *Sicyos* have fleshy (fresh up to *c.* 10 cm long) or hard fruits (5 - 10 mm long) that are smooth or bear barbed or hooked spines (Figs. 1 and S3). Since the spiny *Sicyos* fruits are presented in multi-seeded infructescences, one contact with a bird can easily lead to several seeds being transported. Fruit morphology has been evolutionarily labile, and even fairly large wings have arisen several times (Fig. S3), the latter surprisingly not linked to any long distance dispersal events. All lineages or species involved in long-distance dispersal (Hawaii, the Galápagos, Australia, New Zealand) have spiny fruits. The spines, however, are readily lost; in *S. edulis*, natural populations can contain individuals with spiny or smooth fruits (Lira *et al.*, 1999). Spines were also lost in the Hawaiian clade, where fruits are unarmed or in four species retain stubby outgrowths (Telford, 1990). Loss of dispersal ability is well documented in other insular species (Carlquist, 1966a, b, 1974), the prime example being *Bidens*, which on the Hawaiian Islands lost the barbed awns responsible for dispersal in the mainland species (Carlquist, 1966a, 1967). Selection for loss of dispersibility should be strong since the majority of propagules that are dispersed away from islands will be lost at sea. In Hawaiian *Sicyos*, this limited dispersal ability could have promoted isolation of populations and thus allopatric speciation in the diverse habitats and species build-up.

## Conclusions

*Sicyos*, a genus of 75 species once the names in the segregate genera are transferred, includes four trans-Pacific disjunctions. The importance of small-scale habitat diversity and morphological adaptations (loss of spines leading to reduced dispersal ability) for species accumulation seems to be the strongest signal coming out of this analysis. Reduced dispersal ability may have resulted in isolated populations and allopatric speciation in Hawaii, while the Galápagos and Australian/New Zealand species so far retained spines and high dispersal ability.

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## References

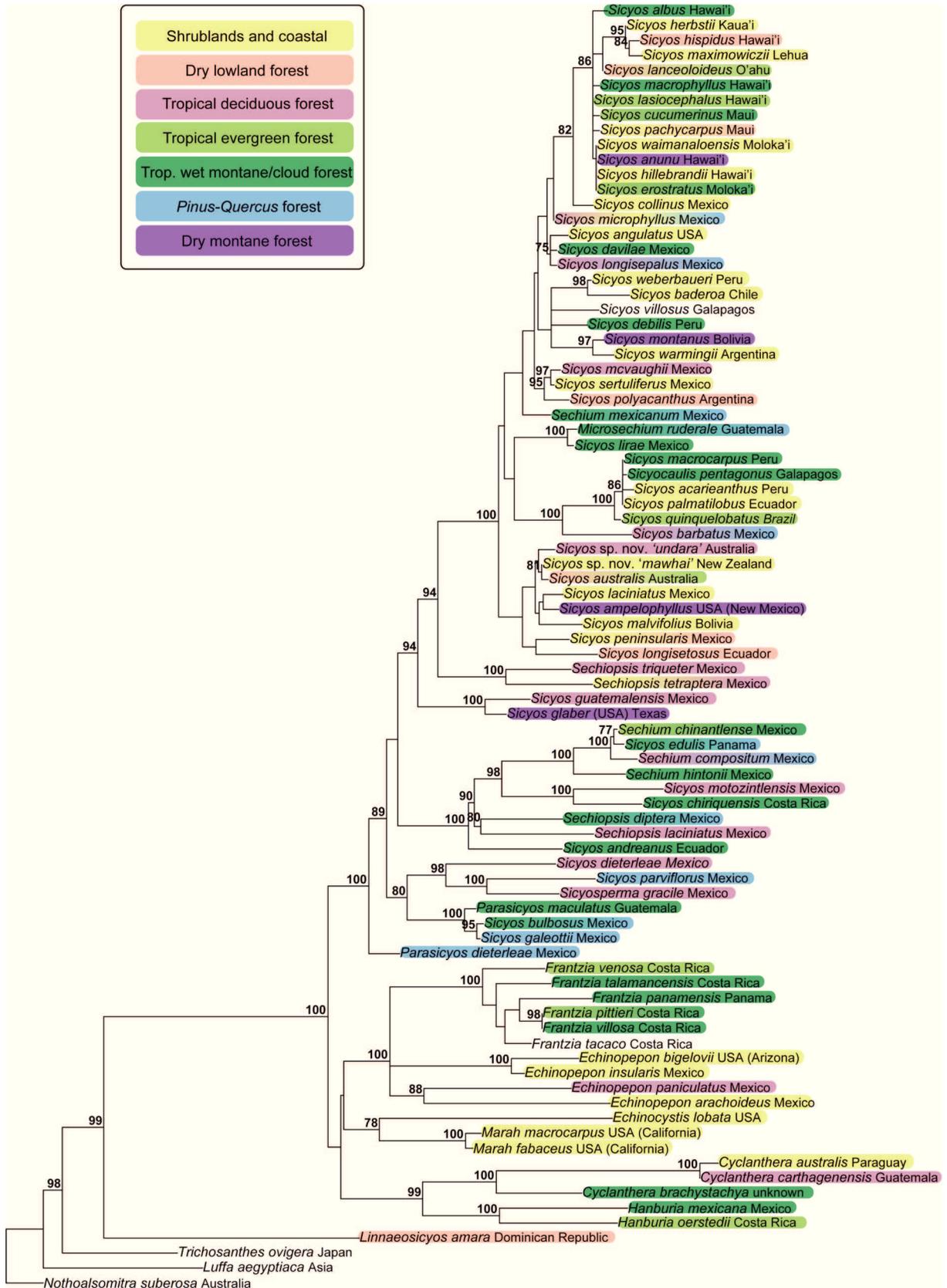
- Baldwin, B.G. & Robichaux, R.H. (1995) Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae): new molecular phylogenetic perspectives. *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (ed. by W.L. Wagner & V.A. Funk), pp. 259-287. Smithsonian Institution Press, Washington, DC.
- Baldwin, B.G. & Wagner, W.L. (2010) Hawaiian angiosperm radiations of North American origin. *Annals of Botany*, **105**, 849–879.
- Bungartz, F., Herrera, H.W., Jaramillo, P., Tirado, N., Jiménez-Uzategui, G., Ruiz, D., Guézou, A. & Ziemmeck, F. (2009) *Charles Darwin Foundation Galapagos Species Checklist*. Charles Darwin Foundation, Puerto Ayora, Galápagos. Available at: <http://www.darwinfoundation.org/datazone/checklists/> (last accessed 10 July 2011).
- Carine, M.A. & Schaefer, H. (2010) The Azores diversity enigma: why are there so few Azorean endemic flowering plants and why are they so widespread? *Journal of Biogeography*, **37**, 77-89.
- Carlquist, S. (1966a) The biota of long-distance dispersal. II. Loss of dispersibility in Pacific Compositae. *Evolution*, **20**, 433–455.
- Carlquist, S. (1966b) The biota of long-distance dispersal. III. Loss of dispersibility in the Hawaiian flora. *Brittonia*, **20**, 30-48.
- Carlquist, S. (1967) The biota of long-distance dispersal. V. Plant dispersal to Pacific Compositae. *Bulletin of the Torrey Botanical Club*, **94**, 129–162.
- Carlquist, S. (1974) *Island Biology*. Columbia University Press, New York.
- Carr, G. (2006) *Hawaiian Native Plant Genera*. University of Hawaii Botany Department, Manoa, Hawaii. Available at: <http://www.botany.hawaii.edu/faculty/carr/natives.htm> (last accessed: 10 July 2011).
- Clark, J.R., Ree, R.H., Alfaro, M.E., King, M.G., Wagner, W.L., & Roalson, E.H. (2008) A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. *Systematic Biology*, **57**, 693–707.
- Clark, J.R., Wagner, W.L. & Roalson, E.H. (2009) Patterns of diversification and ancestral range reconstruction in the southeast Asian-Pacific angiosperm lineage *Cyrtandra* (Gesneriaceae). *Molecular Phylogenetics and Evolution*, **53**, 982-994.
- Cronk, Q.C.B., Kiehn, M., Wagner, W.L. & Smith, J.F. (2005) Evolution of *Cyrtandra* (Gesneriaceae) in the Pacific Ocean: the origin of a supertramp clade. *American Journal of Botany*, **92**, 1017–1024.
- Cuddihy, L.W. (1989) Vegetation zones of the Hawaiian Islands. *Conservation biology in Hawai'i* (ed. By C.P. Stone and D.B. Stone), pp. 27-37. University of Hawaii Press, Honolulu.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Duchen, P. & Renner, S.S. (2010) The evolution of *Cayaponia* (Cucurbitaceae): Repeated shifts from bat to bee pollination and long-distance dispersal to Africa 2-5 million years ago. *American Journal of Botany*, **97**, 1129–1141.
- Falla, R.A. (1960) Birds as dispersal agents. *Proceedings of the Royal Society of London, B: Biological Sciences*, **152**, 655-659.
- Federov, A.V., Dekens, P.S., McCarthy, M., Ravelo, A.C., deMenocal, P.B., Barreiro, M., Pacanowski, R.C. & Philander, S.G. (2006) The Pliocene paradox (Mechanisms for a Permanent El Niño), *Science*, **312**, 1485-1489.
- Harbaugh, D.T. & Baldwin, B.G. (2007) Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersal throughout the Pacific. *American Journal of Botany*, **94**, 1028–1040.

- Harbaugh, D.T., Wagner, W.L., Allan, G.J. & Zimmer, E.A. (2009) The Hawaiian Archipelago is a stepping stone for dispersal in the Pacific: an example from the plant genus *Melicope* (Rutaceae). *Journal of Biogeography*, **36**, 230–241.
- Harmon, L.J., Weir, J.T., Brock, C., Glor, R.E. & Challenger, W. (2008) GEIGER: investigating evolutionary radiations. *Bioinformatics*, **24**, 129–131.
- Havran, J.C., Sytsma, K.J., & Ballard Jr., H.E. (2009) Evolutionary relationships, interisland biogeography and molecular evolution in the Hawaiian violets (*Viola*: Violaceae). *American Journal of Botany*, **96**, 2087–2099.
- Jeffrey, C. & Trujillo, B. (1992) Cucurbitaceae. *Flora of Venezuela*. vol V, part 1 (ed. By G. Morillo), pp. 11–202. Fondo Editorial Acta Científica Venezolana, Caracas.
- Keppel, G., Lowe, A.J. & Possingham, H.P. (2009) Changing perspectives on the biogeography of the tropical South Pacific: influences of dispersal, vicariance and extinction. *Journal of Biogeography*, **36**, 1035–1054.
- Kocyan, A., Zhang, L.-B., Schaefer, H. & Renner, S.S. (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553–577.
- LaBerge, W.E. & Hurr, P.D. (1965) A new subgenus and species of maternal *Andrena* from the flowers of *Sicyos* (Cucurbitaceae) in Mexico. *Pan-Pacific Entomologist*, **41**, 186–193.
- Lemey, P., Rambaut, A., Drummond, A.J. & Suchard, M.A. (2009) Bayesian phylogeography finds its roots. *PLoS Computational Biology*, **5**: e1000520.
- Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, **50**, 913–925.
- Lindqvist, C. & Albert, V.A. (2002) Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany*, **89**, 1709–1724.
- Lindqvist, C., Motley, T.J., Jeffrey, J.J. & Albert, V.A. (2003) Cladogenesis and reticulation in the Hawaiian endemic mints (Lamiaceae). *Cladistics*, **19**, 480–495.
- Lira, R. (1994) Especie nueva de *Microsechium* (Cucurbitaceae, tribu Sicyoeae, subtribu Sicyinae) del estado de Oaxaca, México. *Anales del Instituto de Biología de la Universidad Nacional Autónoma de México, Serie Botánica*, **65**, 73–81.
- Lira, R. & Rodríguez-Arévalo, I. (1999) Nuevas especies de los géneros *Cyclanthera* Schrad. y *Sicyos* L. (Sicyoeae, Cucurbitaceae) para la flora de México. *Acta Botanica Mexicana*, **48**, 11–19.
- Lira, R., Castrejón, J., Zamudio, S. & Rojas-Zenteno, C. (1999) Propuesta de ubicación taxonómica para los chayotes silvestres (*Sechium edule*, Cucurbitaceae) de México. *Acta Botánica Mexicana*, **49**, 47–61.
- Macbride, F.J. (1960) *Flora of Peru*. *Botanical Series of the Field Museum of Natural History*, Vol. XIII, Part V, No. 2. Chicago Natural History Museum Press, Chicago.
- Maddison, D.R. & Maddison, W.P. (2003) *MacClade*, version 4.0.8. Sinauer Associates, Sunderland, MA.
- Maddison, W.P. & Maddison, D.R. (2009) *MESQUITE: A modular system for evolutionary analysis*, version 2.72. Available at: <http://mesquiteproject.org/mesquite/mesquite.html> (last accessed 10 July 2011).
- Magallón, S. & Sanderson, M.J. (2001) Absolute diversification rates in angiosperm clades. *Evolution*, **55**, 1762–1780.
- Marks, J.S. & Leasure, S.M. (1992) Breeding biology of Tristram's storm-petrel on Laysan Island. *Wilson Bulletin*, **104**, 719–731.
- Mueller-Dombois, D. & Fosberg, F.R. (1998) *Vegetation of the tropical Pacific islands*. Springer, Berlin.
- Mummenhoff, K., Linder, P., Friesen, N., Bowman, J.L., Lee, J.-Y. & Franzke, A. (2004) Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium*

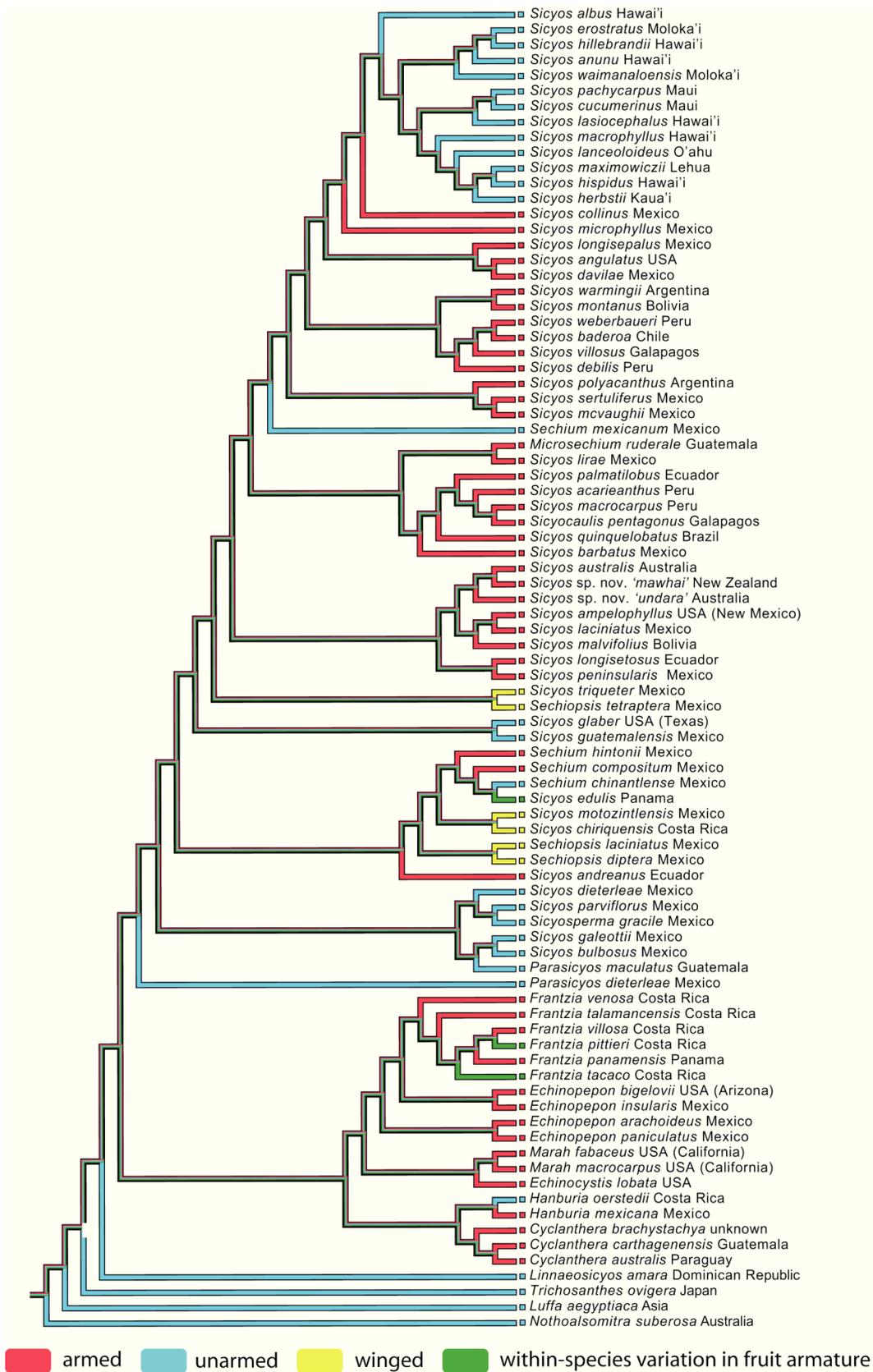
- sensu stricto (Brassicaceae) species from Australia and New Zealand. *American Journal of Botany*, **91**, 254–261.
- Pole, M. (1994) The New Zealand flora - entirely long-distance dispersal? *Journal of Biogeography*, **21**, 625–635.
- Price, J.P. & Clague, D.A. (2002) How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 2429–2435.
- Rambaut, A. (2006) *FigTree* v. 1.3.1. Available at: <http://tree.bio.ed.ac.uk/software/figtree/> (last accessed August 2011).
- Rambaut, A. & Drummond, A.J. (2007) *Tracer* 1.5. Available at : <http://beast.bio.ed.ac.uk/Tracer> (last accessed August 2011)
- Rodríguez-Arévalo, I. (2003) A new species of *Sicyos* (Cucurbitaceae, Sicyoeae, Sicyinae) from Mexico and Guatemala. *Brittonia*, **55**, 69–72.
- Rodríguez-Arévalo, I. & Lira, R. (2001) Nueva especie del género *Sicyos* L. (Cucurbitaceae) para la República Mexicana. *Boletín de la Sociedad Botánica de México*, **68**, 81–84.
- Rodríguez-Arévalo, I., Lira, R. & Dávila, P. (2004) Two new species of *Sicyos* L. (Cucurbitaceae) from Guerrero and Oaxaca, Mexico. *Botanical Journal of the Linnean Society*, **145**, 373–378.
- Rodríguez-Arévalo, I., Lira, R. & Calzada, I. (2005) A new species of *Sicyos* L. (Cucurbitaceae) from Oaxaca, Mexico. *Brittonia*, **57**, 43–46.
- Salard-Chebouldaef, M. (1978) Sur la palynoflore Maestrichtienne et Tertiaire du bassin sédimentaire littoral du Cameroun. *Pollen et Spores*, **20**, 215–260.
- Schaefer, H. & Renner, S.S. (2010) A gift from the New World? The West African crop *Cucumeropsis mannii* and the American *Posadaea sphaerocarpa* (Cucurbitaceae) are the same species. *Systematic Botany*, **35**, 534–540.
- Schaefer, H. & Renner, S.S. (2011a) Cucurbitaceae. *Families and Genera of Vascular Plants* Vol. 10. (ed. by K. Kubitzki), pp. 112–174. Springer Verlag, Berlin.
- Schaefer, H. & Renner, S.S. (2011b) Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon*, **60**, 122–138.
- Schaefer, H., Kocyan, A., & Renner, S.S. (2008a). *Linnaeosicyos* (Cucurbitaceae), a new genus for the Caribbean sister group of the New World Sicyeae. *Systematic Botany* **33**, 349–355.
- Schaefer, H., Telford, I.R.H., & Renner, S.S. (2008b) *Austrobryonia* (Cucurbitaceae), a new Australian endemic genus, is the closest living relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*. *Systematic Botany*, **33**, 125–132.
- Schaefer H., Heibl, C. & Renner, S.S. (2009) Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 843–851.
- Schaefer, H., Moura, M., Maciel, M.G.B., Silva, L., Rumsey, F.J. & Carine, M.A. (2011) The Linnean shortfall in oceanic island biogeography: a case study in the Azores. *Journal of Biogeography*, **38**, 1345–1355.
- Schilling, E.E., Panero, J.L. & Eliasson, U.H. (1994) Evidence from chloroplast DNA restriction site analysis on the relationships of *Scalesia* (Asteraceae: Heliantheae). *American Journal of Botany*, **8**, 248–254.
- Sebastian, P., Schaefer, H. & Renner, S.S. (2010a) Darwin's Galapagos gourd: providing new insights 175 years after his visit. *Journal of Biogeography*, **37**, 975–980.
- Sebastian, P., Schaefer, H., Telford, I.R.H. & Renner, S.S. (2010a) Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences USA*, **107**, 14269–14273.

- Stamatakis A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688-2690.
- Starr, F. & Martz, K. (1999) *S.S. Midway Expedition – May 21, 1999-June 16, 1999. Trip Report*. United States Fish and Wildlife Service. Available at: <http://www.hear.org/starr/publications/ssmidway.pdf> (last accessed: Aug. 2011)
- Telford, I.R.H. (1990) Cucurbitaceae. *Manual of the Flowering Plants of Hawai'i* (ed. by W.L. Wagner, D.R. Herbst and S.H. Sohmer) pp. 568–581. University of Hawaii Press & Bishop Museum Press, Honolulu.
- Telford, I.R.H., Sebastian, P., Bruhl, J.J., & Renner, S.S. (2011) *Cucumis* (Cucurbitaceae) in Australia and eastern Malaysia, including newly recognized species and the sister species to *C. melo*, *Systematic Botany*, **36**, 376–389.
- Telford, I.R.H., Sebastian, P., Bruhl, J.J. & Renner, S.S. *Sicyos* (Cucurbitaceae) in Australia, New Zealand and the islands of the south-western Pacific. In review.
- Tomkins, R.J. (1982) Migration of White-faced Storm-Petrels *Pelagodroma marina* in the South Pacific and the status of the Kermadec subspecies. *Emu*, **84**, 32–35.
- Valente, L.M., Savolainen, V. & Vargas, P. (2010) Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1489–1496.
- Volz, S.M., & Renner, S.S. (2009) Phylogeography of the ancient Eurasian medicinal plant genus *Bryonia* (Cucurbitaceae) inferred from nuclear and chloroplast sequences. *Taxon*, **58**, 550-560.
- Wagner, W.L. & Shannon, R.K. (1999) Nomenclator of Hawaiian *Sicyos* (Cucurbitaceae). *Novon*, **9**, 441-447.
- Wagner, W.L., Herbst D.R. & Sohmer S.H. (1990) *Manual of the Flowering Plants of Hawai'i*. University of Hawaii Press & Bishop Museum Press, Honolulu.
- Wara, M.W., Ravelo, A.C. & Delaney, M.L. (2005) Permanent El Niño-like conditions during the Pliocene warm period. *Science*, **309**, 758.
- Weeks, A., Baird, K.E. & McMullen, C.K. (2010) Origin and evolution of endemic Galápagos *Varronia* species (Cordiaceae). *Molecular Phylogenetics and Evolution*, **57**, 948–954.
- Wiggins, I.L. & Porter, D.M. (1971) *Flora of the Galapagos Islands*. Stanford University Press, Stanford, CA.
- Willyard, A., Wallace, L.E., Wagner, W.L., Weller, S.G., Sakai, A.K. & Nepokroeff, M. (2011) Estimating the species tree for Hawaiian *Schiedea* (Caryophyllaceae) from multiple loci in the presence of reticulate evolution. *Molecular Phylogenetics and Evolution*, **60**, 29–48.
- Wright, S.D., Yong, C.G., Dawson, J.W., Whittaker, D.J. & Gardner, R.C. (2000) Riding the ice age El Niño? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros* (Myrtaceae) inferred from nuclear ribosomal DNA. *Proceedings of the National Academy of Sciences USA*, **97**, 4118–4123.





**Fig. S2.** Habitat types of extant Sicyoeae species plotted on an 86-species maximum likelihood phylogeny. Colour codes for character states are as stated in the legend.



**Fig. S3.** Shifts between armed fruits and unarmed fruits during the evolution of Sicyoeae inferred on an 86-species maximum likelihood phylogeny under maximum likelihood optimization. Characters were coded as indicated in the legend.

**Table S1:** Species names with their authors, geographic origin of the sequenced sample, voucher deposition, and GenBank accession numbers for all sequences included into this study (*italics* denote sequences that were not used for the final dataset). Herbarium acronyms follow the *Index Herbariorum* at <http://sciweb.nybg.org/science2/>

Species	DNA source	Geographic origin of the sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>psbA-trnH</i> spacer	<i>rbcL</i> gene
<i>Cyclanthera australis</i> Cogn. ( <i>Pseudocyclanthera australis</i> (Cogn.) Mart. Corv.)	E. de Paula 1834 (K)	Paraguay			EF066330	EF066336			
<i>Cyclanthera brachystachia</i> (Ser.) Cogn.	S.S. Renner et al. 2767 (M)	cult. Mainz BG		DQ536767	DQ536767	DQ648172			DQ535749
<i>Cyclanthera carthagenensis</i> (Jacq.) H.Schaef. & S.S. Renner ( <i>Rytidostylis carthagenensis</i> (Jacq.) Kuntze)	S.S. Renner & A. Kocyan 2752 (M)	Guatemala	JN560181	DQ536776	DQ536776	DQ648188	JN560559	JN560279	DQ535841
<i>Echinocystis lobata</i> (Michx.) Torr. & A.Gray	S.S. Renner 2829 (M)	USA		DQ536814	DQ536814	DQ648174			DQ535809
<i>Echinopepon arachioideus</i> (Dieterle) A.H. Monro & P.J. Stafford	Machuca 6547 (MEXU)	Mexico	JN560182	DQ536786	DQ536786	DQ536529	JN560560	JN560280	DQ535739
<i>Echinopepon bigelovii</i> (S.Watson) S. Watson	J. Buegge 1182 (ASU)	USA: Arizona		DQ536790	DQ536790	DQ648156			JN560636
<i>Echinopepon insularis</i> S. Watson ( <i>Vaseyanthus insularis</i> Rose)	E.J. Lott & T.H. Atkinson 2428 (MO)	Mexico: Isla San Pedro de Martir		JN560465	JN560465	JN560381	JN560561	JN560281	JN560637
<i>Echinopepon paniculatus</i> (Cogn.) Dieterle	R. Torres C. 14047 (M)	Mexico	JN560183	DQ536815	DQ536815	DQ536548	JN560562	JN560282	DQ535810
<i>Frantzia panamensis</i> Wunderlin ( <i>Sechium panamense</i> (Wunderlin) Lira & F. Chiang)	B. Hamel 7365 (MEXU)	Panama: Chiriqui		JN560466	JN560466				
<i>Frantzia pittieri</i> (Cogn.) Pittier ( <i>Sechium pittieri</i> (Cogn.) C. Jeffrey)	B. Hamel et al. 25338 (MO)	Costa Rica	JN560184	JN560467	JN560467	JN560382		JN560283	

Species	DNA source	Geographic origin of the sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>psbA-trnH</i> spacer	<i>rbcL</i> gene
<i>Frantzia tacaco</i> (Pittier) Wunderlin ( <i>Sechium tacaco</i> Pittier)	R. Lira et al. 1062 (MEXU)	Costa Rica: San Jose	JN560186	JN560469	JN560469	JN560383	JN560563	JN560285	JN560638
<i>Frantzia tacaco</i> (Pittier) Wunderlin ( <i>Sechium tacaco</i> Pittier)	P. Döbbeler 3787 (M)	Costa Rica: Alajuela	<i>JN560185</i>	<i>JN560468</i>	<i>JN560468</i>	<i>EU436327</i>		<i>JN560284</i>	<i>EU436380</i>
<i>Frantzia talamancensis</i> Wunderlin ( <i>Sechium talamancense</i> (Wunderlin) C. Jeffrey)	J.E.. Poppleton & A.G. Shuey 551 (USF)	Costa Rica: San Jose	JN560187		JN560470	JN560384		JN560286	
<i>Frantzia venosa</i> L.D. Gómez ( <i>Sechium venosum</i> (L.D. Gómez) Lira & F. Chiang)	R. Lira & R. Ocampo 1036 (K)	Costa Rica: Limón	JN560188	JN560471	JN560471	JN560385		JN560287	JN560639
<i>Frantzia villosa</i> Wunderlin ( <i>Sechium villosum</i> (Wunderlin) C. Jeffrey)	W.C. Burger & J.L. Gentry Jr. 9227 (NY)	Costa Rica: Alajuela			JN560472	JN560386		JN560288	
<i>Hanburia mexicana</i> Seem.	F. Ventura A. 15150 (MO)	Mexico: Veracruz		DQ536825	DQ536825	JN560387	JN560564	JN560289	DQ535756
<i>Hanburia oerstedii</i> (Cogn.) H.Schaef. & S.S. Renner ( <i>Elateriopsis oerstedii</i> Cogn.) Pittier)	A. Jiménez M. 3961-A (G)	Costa Rica	JN560189	JN560473	JN560473	DQ536551		JN560290	DQ535752
<i>Linnaeosicyos amara</i> (L.) H.Schaef. & Kocyan ( <i>Trichosanthes amara</i> L.)	M. Mejia et al. 1877 (NY)	Dominican Republic	JN560190	DQ536873	DQ536873	DQ536602	JN560565	JN560291	DQ535774
<i>Luffa aegyptiaca</i> Mill.	leg. L.X. Zhou	cult. Guangzhou BG		DQ536836	DQ536836	DQ536564			DQ535827
<i>Luffa aegyptiaca</i> Mill.	S.S. Renner et al. 2783 (M)	cult. Mainz BG	JN560191				JN560566	JN560292	
<i>Marah fabaceus</i> (Naud.) Greene	R. Ricklefs & S.S. Renner 1 (MO)	USA: California		DQ536837	DQ536837	AY973021			DQ535758
<i>Marah macrocarpus</i> Greene	D. Arisa & S. Swensen 1009 (RSA)	Sonoran Desert							AY968524
<i>Marah macrocarpus</i> Greene	M. Olson s.n. (MO)	Sonoran Desert	JN560192	AY968571	AY968387	DQ536566	JN560567	JN560293	
<i>Microsechium gonzalo-palomae</i> Lira	R. Lira 1230 type (MEXU)	Mexico: Oaxaca	JN560193	JN560474	JN560474		JN560568	JN560294	JN560640

<i>Microsechium gonzalo-palomae</i> Lira	R. Lira 1230 isotype (K)	Mexico: Oaxaca	JN560194						
<i>Microsechium ruderale</i> Naudin	H. Förther 10430 (MSB)	Guatemala: Alta Verapaz	JN560195	DQ536840	DQ536840	DQ536570	JN560569	JN560295	
<i>Microsechium ruderale</i> Naudin.	R. Lira 1077 MEXU)	Mexico: México	<i>JN560196</i>	<i>JN560475</i>	<i>JN560475</i>	<i>JN560388</i>		<i>JN560296</i>	<i>JN560641</i>
<i>Nothoalsomitra suberosa</i> (F.M. Bailley) I. Telford, GT	I.R.H. Telford 12487 (NE)	Australia: QLD		DQ536844	DQ536844	DQ536575			DQ535762
<i>Parasicyos dieterleae</i> Lira & R. Torres	A. Garcia M. et al. 1704 (MO)	Mexico: Oaxaca	JN560197	DQ536846	DQ536846	DQ536577	JN560570	JN560297	DQ535763
<i>Parasicyos dieterleae</i> Lira & R. Torres	Tenoris 17076 (MEXU)	Mexico: Oaxaca	<i>JN560198</i>	<i>JN560476</i>	<i>JN560476</i>	<i>JN560389</i>	<i>JN560571</i>	<i>JN560298</i>	<i>JN560642</i>
<i>Parasicyos maculatus</i> Dieterle	collector unknown (M)	Guatemala: Alta Verapaz	<i>JN560199</i>	<i>JN560477</i>	<i>JN560477</i>	<i>JN560390</i>	JN560572	<i>JN560299</i>	JN560643
<i>Parasicyos maculatus</i> Dieterle	C.A. Todzia et al. 2840 (NY)	Mexico: Oaxaca	JN560200	JN560478	JN560478	JN560391		JN560300	
<i>Sechiopsis diptera</i> Kearns	E. Martinez 22147 (MEXU)	Mexico: Chiapas	JN560201	JN560479	JN560479	JN560392	JN560573	JN560301	JN560644
<i>Sechiopsis laciniatus</i> Brandegee ( <i>Pterosicyos laciniatus</i> Brandegee (Kearns))	M. Nee 32356 (MO)	Mexico: Chiapas	JN560202	JN560480	JN560480	JN560393	JN560574	JN560302	
<i>Sechiopsis tetraptera</i> Dieterle	J. Calónico Soto 4793 (M)	Mexico: Jalisco	JN560203	DQ536860	DQ536860	DQ536588	JN560575	JN560303	DQ535842
<i>Sechiopsis tetraptera</i> Dieterle	R. Lira 1315 (MEXU)	Mexico: Michoacán	JN560204	JN560481	JN560481	JN560394	JN560576	JN560304	JN560645
<i>Sechium chinantlense</i> R. Lira & F. Chiang	R. Lira 1187 (MEXU)	Mexico: Oaxaca	JN560206	JN560483	JN560483	JN560396	JN560578	JN560306	JN560647
<i>Sechium compositum</i> (Donn.Sm.) C. Jeffrey ( <i>Microsechium compositum</i> Donn. Sm.)	R. Lira 960 (MEXU)	Mexico: Chiapas	JN560208	JN560485	JN560485	JN560398	JN560579	JN560307	JN560648
<i>Sechium compositum</i> (Donn.Sm.) C. Jeffrey ( <i>Microsechium compositum</i> Donn. Sm.)	R. Lira 1289 (MEXU)	Mexico: Chiapas	<i>JN560207</i>	<i>JN560484</i>	<i>JN560484</i>	<i>JN560397</i>			

Species	DNA source	Geographic origin of the sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>psbA-trnH</i> spacer	<i>rbcL</i> gene
<i>Sechium hintonii</i> (Paul G. Wilson) C. Jeffrey ( <i>Microsechium hintonii</i> Paul G. Wilson)	R. Cuevas 3247 (MEXU)	Mexico: Jalisco	JN560210	JN560487	JN560487				
<i>Sechium hintonii</i> (Paul G. Wilson) C. Jeffrey ( <i>Microsechium hintonii</i> Paul G. Wilson)	R. Lira 1082 (MEXU)	Mexico: México	JN560211	JN560488	JN560488	JN560400		JN560309	JN560649
<i>Sechium hintonii</i> (Paul G. Wilson) C. Jeffrey ( <i>Microsechium hintonii</i> Paul G. Wilson)	R. Lira 1313 (MEXU)	Mexico: Guerrero	JN560212	JN560489	JN560489	JN560401	JN560581	JN560310	
<i>Sechium mexicanum</i> Lira & M. Nee	R. Lira & M. Nee 1368 (MEXU)	Mexico: Querétaro				JN560402	JN560582	JN560311	JN560650
<i>Sechium mexicanum</i> Lira & M. Nee	M. Nee 32886 (K)	Mexico: Veracruz	JN560213	JN560490	JN560490	JN560403		JN560312	JN560651
<i>Sicyocaulis pentagonus</i> Wiggins	H.H. van der Werff 1394 (CAS)	Galapagos (Ecuador): Santa Cruz	JN560214	JN560491	JN560491	JN560404		JN560313	JN560652
<i>Sicyos acarieanthus</i> Harms	M. Weigend et al. 8542 (M)	Peru: Lambayeque	JN560215	JN560492	JN560492	JN560405	JN560583	JN560314	JN560653
<i>Sicyos albus</i> (H.St.John) I.Telford ( <i>Sarx alba</i> H.St.John)	K.R. Wood 2595 (US)	Hawaii: Hawai'i	JN560216	JN560493	JN560493	JN560406	JN560584	JN560315	JN560654
<i>Sicyos ampelophyllus</i> Wooton & Standl.	R. Spellenberg 6866 (MO)	USA: New Mexico	JN560218	JN560495	JN560495	JN560408	JN560585	JN560317	JN560655
<i>Sicyos andreanus</i> Cogn.) ( <i>Anomalosicyos andreanus</i> (Cogn.) Gentry)	J. Ramos et al. 7050 (NY)	Ecuador: Cotopaxi			JN560496	JN560409		JN560318	
<i>Sicyos andreanus</i> Cogn.) ( <i>Anomalosicyos andreanus</i> (Cogn.) Gentry)	P. Silverstone-Sopkin et al. 9471 (NY)	Ecuador: Cotopaxi		JN560497	JN560497	JN560410	JN560586	JN560319	
<i>Sicyos angulatus</i> L.	M.W. Chase 979 (K)	North America	JN560219	DQ536777	DQ536777	DQ648189			DQ535847
<i>Sicyos ampelophyllus</i> Wooton & Standl.	R. Spellenberg 10624 (NY)	USA: New Mexico	JN560217	JN560494	JN560494	JN560407		JN560316	

<i>Sicyos anumu</i> (H.St.John) I.Telford	I.R.H. Telford 10276 (CANB)	Hawaii: Hawai'i	JN560220	JN560498	JN560498	JN560411	JN560587	JN560320	JN560656
<i>Sicyos australis</i> Endl.	J. Dare 139 (NE)	Australia: QLD	<i>JN560221</i>	<i>JN560499</i>	<i>JN560499</i>	<i>JN560412</i>	<i>JN560588</i>	<i>JN560321</i>	<i>JN560657</i>
<i>Sicyos australis</i> Endl.	I.R.H. Telford 12575 (NE)	Australia: NSW	JN560222	JN560500	JN560500	JN560413	JN560589	JN560322	JN560658
<i>Sicyos baderoa</i> Hook. & Arn.	C. Heibl 01 045 (M)	Chile: Antofagasta	JN560223	DQ536866	DQ536866	DQ536594	JN560590	JN560323	DQ535848
<i>Sicyos baderoa</i> Hook. & Arn.	L.R. Landrum 7505 (MO)	Chile: Coquimbo	<i>JN560224</i>	<i>JN560501</i>	<i>JN560501</i>	<i>JN560414</i>	<i>JN560591</i>	<i>JN560324</i>	<i>JN560659</i>
<i>Sicyos barbatus</i> (Gentry) C.Jeffrey ( <i>Anomalosicyos</i> <i>barbatus</i> Gentry)	J.V. A. Dieterle 4102 (NY)	Mexico: Jalisco	JN560225	JN560502	JN560502	JN560415		JN560325	
<i>Sicyos bulbosus</i> Rodr.-Arév., Lira & Dávila	S. Figueroa & F.Y. Guzman 281 (MEXU)	Mexico: Oaxaca	JN560226	JN560503	JN560503	JN560416	JN560592	JN560326	JN560660
<i>Sicyos chiriquensis</i> Hammel & D'Arcy ( <i>Costarica hamata</i> Hammel & D'Arcy)	B. Hammel et al. 25339 (MO)	Costa Rica: Cartago	<i>JN560179</i>	<i>JN560463</i>	<i>JN560463</i>	<i>JN560379</i>		<i>JN560277</i>	<i>JN560634</i>
<i>Sicyos chiriquensis</i> Hammel & D'Arcy ( <i>Costarica hamata</i> Hammel & D'Arcy)	A. Rodriguez et al. 2006 (MO)	Costa Rica: Cartago	JN560180	JN560464	JN560464	JN560380	JN560558	JN560278	JN560635
<i>Sicyos collinus</i> B.L.Rob. & Fernald	C.V. Hartman 773 (HUH)	Mexico: Chihuahua	JN560227	JN560504	JN560504			JN560327	
<i>Sicyos cucumerinus</i> A.Gray	H.L. Oppenheimer & S. Perlman H30803 (BISH)	Hawaii: Maui	JN560229	JN560506	JN560506	JN560418	JN560594	JN560329	JN560662
<i>Sicyos cucumerinus</i> A.Gray	H.L. Oppenheimer & S. Perlman H110625 (BISH)	Hawaii: Moloka'i	<i>JN560228</i>	<i>JN560505</i>	<i>JN560505</i>	<i>JN560417</i>	<i>JN560593</i>	<i>JN560328</i>	JN560661
<i>Sicyos davilae</i> Rodr.-Arév. & Lira	R. Lira et al. 949 (MEXU)	Mexico: Chiapas	JN560230	JN560507	JN560507	JN560419	JN560595	JN560330	JN560663
<i>Sicyos debilis</i> Cogn.	J.D. Boeke 1993 (NY)	Peru: Amazonas	JN560231	JN560508	JN560508	JN560420		JN560331	
<i>Sicyos dieterleae</i> Rodr.-Arév. & Lira	R. Lira et al. 1385 (MEXU)	Mexico: Michoacán	JN560232	JN560509	JN560509	JN560421	JN560596	JN560332	JN560664
<i>Sicyos edulis</i> Jacq. ( <i>Sechium</i> <i>edule</i> (Jacq) Sw.)	M. Nee & T. Andres 46304 (NY)	Panama: Chiriquí	JN560209	JN560486	JN560486	JN560399	JN560580	JN560308	
<i>Sicyos edulis</i> Jacq. ( <i>Sechium</i> <i>edule</i> (Jacq) Sw.)	M. Olson 832 (MEXU)	Mexico		<i>DQ536861</i>	<i>DQ536861</i>	<i>DQ536589</i>			DQ535843

Species	DNA source	Geographic origin of the sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>psbA-trnH</i> spacer	<i>rbcL</i> gene
<i>Sicyos erostratus</i> H.St.John	K.R. Wood, J. Lau 2898 (US)	Hawaii: Moloka'i	JN560233	JN560510	JN560510	JN560422	JN560597	JN560333	JN560665
<i>Sicyos galeottii</i> Cogn.	D.H. Lorence & R. Cedillo 634 (K)	Mexico: Oaxaca	JN560234	JN560511	JN560511	JN560423	JN560598	JN560334	
<i>Sicyos galeottii</i> Cogn.	I. Rodriguez & A. Lecona 270 (MEXU)	Mexico: Oaxaca		<i>JN560512</i>	<i>JN560512</i>	<i>JN560424</i>	<i>JN560599</i>	<i>JN560335</i>	JN560666
<i>Sicyos glaber</i> Wooton	R.D. Worthington 12743 (NY)	USA: Texas	JN560235	JN560513	JN560513	JN560425	JN560600		
<i>Sicyos guatemalensis</i> Standl. & Steyerl.	I. Rodriguez 260 (MEXU)	Mexico: Oaxaca	JN560236	JN560514	JN560514	JN560426	JN560601	JN560336	JN560667
<i>Sicyos herbstii</i> (H.St.John) I.Telford	T. Flynn et al. 4438 (US)	Hawaii: Kaua'i	JN560237	JN560515	JN560515	JN560427	JN560602	JN560337	JN560668
<i>Sicyos hillebrandii</i> H.St.John	O. Degener 21364 (M)	Hawaii (?)	JN560238	EU436370	JN560516	EU436342	JN560603	JN560338	EU436394
<i>Sicyos hillebrandii</i> H.St.John	T. Flynn et al. 2285 (MO)	Hawaii: Hawai'i	JN560239	JN560517	JN560517	JN560428	JN560604	JN560339	JN560669
<i>Sicyos hispidus</i> Hillebr.	I.R.H. Telford 10281 (CANB)	Hawaii: Hawai'i	JN560240	JN560518	JN560518	JN560429	JN560605	JN560340	JN560670
<i>Sicyos laciniatus</i> L.	J.V.A. Dieterle 3405 (NY)	Mexico: San Luis Potosi	JN560241	JN560519	JN560519	JN560430	JN560606	JN560341	
<i>Sicyos laciniatus</i> L.	J. Hendrickson & E. Lee 17532 (HUH)	Mexico: San Luis Potosi	<i>JN560242</i>		<i>JN560520</i>			<i>JN560342</i>	
<i>Sicyos laciniatus</i> L.	I. Rodriguez et al. 233 (MEXU)	Mexico: México		<i>JN560521</i>	<i>JN560521</i>				
<i>Sicyos lanceoloideus</i> (H.St.John) W.L.Wagner & D.R.Herbst ( <i>Sicyocarya lanceoloidea</i> H.St.John)	S. Perlman & T. Wood 17215 (US)	Hawaii: O'ahu	<i>JN560243</i>	<i>JN560522</i>	<i>JN560522</i>			<i>JN560343</i>	<i>JN560671</i>
<i>Sicyos lanceoloideus</i> (H.St.John) W.L.Wagner & D.R.Herbst ( <i>Sicyocarya lanceoloidea</i> H.St.John)	I.R.H. Telford 10206 (CANB)	Hawaii: O'ahu	JN560244	JN560523	JN560523	JN560431	JN560607	JN560344	JN560672

<i>Sicyos lasiocephalus</i> Skottsberg ( <i>Skottsbergiana lasiocephala</i> (Skottsberg) H. St. John)	I.R.H. Telford 10283 (CANB)	Hawaii: Hawai'i	JN560245	JN560524	JN560524	JN560432	JN560608	JN560345	JN560673
<i>Sicyos lirae</i> Rodr.-Arév.	G.L. Webster et al. 12823 (MO)	Guatemala: Sacatepéquez	JN560246	JN560525	JN560525	JN560433		JN560346	JN560674
<i>Sicyos longisepalus</i> Cogn.	R. Lira 1372 (MEXU)	Mexico: Querétaro	JN560247	JN560526	JN560526	JN560434	JN560609	JN560347	JN560675
<i>Sicyos longisepalus</i> Cogn.	I. Rodriguez et al. 238 (MEXU)	Mexico: Nayarit				<i>JN560435</i>	<i>JN560610</i>	<i>JN560348</i>	<i>JN560676</i>
<i>Sicyos longisetosus</i> Cogn.	G.P. Lewis & B.B. Klitgaard 3341 (NY)	Ecuador: Manabi	JN560248	JN560527	JN560527	JN560436	JN560611	JN560349	
<i>Sicyos macrocarpus</i> Cogn. ( <i>Anomalosicyos macrocarpus</i> (Cogn.) Gentry)	M.O. Dillon et al. 4304 (K)	Peru: Cajamarca		JN560528	JN560528			JN560350	
<i>Sicyos macrophyllus</i> A.Gray ( <i>Sicyocarya macrophylla</i> (A.Gray) H. St. John)	W.L. Wagner 5578 (CANB)	Hawaii: Hawai'i	JN560249	JN560529	JN560529	JN560437	JN560612	JN560351	JN560677
<i>Sicyos malvifolius</i> Griseb.	A. Burkart 7785 (K)	Argentina: Córdoba	JN560250	JN560530	JN560530	JN560438	JN560613	JN560352	
<i>Sicyos malvifolius</i> Griseb.	W.J. Eyerdam 24930 (HUH)	Bolivia: Cochabamba	<i>JN560251</i>	<i>JN560531</i>	<i>JN560531</i>			<i>JN560353</i>	JN560678
<i>Sicyos mawhai</i> I.Telford & P.Sebastian sp. nov. ined.	E.K. Cameron 6708 (CANB)	New Zealand: North Island, Auckland	<i>JN560252</i>	<i>JN560532</i>	<i>JN560532</i>	<i>JN560439</i>	<i>JN560614</i>	<i>JN560354</i>	<i>JN560679</i>
<i>Sicyos mawhai</i> I.Telford & P.Sebastian sp. nov. ined.	W.R. Sykes 1523 (NE)	New Zealand: Kermadec Islands -	JN560253	JN560533	JN560533	JN560440	JN560615	JN560355	JN560680
<i>Sicyos maximowiczii</i> Cogn. ( <i>Cladocarpa maximowiczii</i> (Cogn.) H. St. John)	S. Perlman & T. Flynn 12487 (US)	Hawaii: Lehua	JN560254	JN560534	JN560534	JN560441	JN560616	JN560356	JN560681
<i>Sicyos mcvaughii</i> Rodr.-Arév., Lira & Calzada	I. Rodriguez 297 (IZTA)	Mexico: Oaxaca	JN560255	JN560535	JN560535	JN560442	JN560617	JN560357	JN560682
<i>Sicyos microphyllus</i> Kunth	I. Rodriguez et al. 254 (MEXU)	Mexico: Michoacán	JN560256	JN560536	JN560536	JN560443	JN560618	JN560358	JN560683
<i>Sicyos montanus</i> Griseb.	J. Steinbach 9851 (HUH)	Bolivia: Cochabamba	JN560257	JN560537	JN560537			JN560359	JN560684
<i>Sicyos motozintlensis</i> E.J.Lott & Fryxell	R. Lira et al. 951 (MEXU)	Mexico: Chiapas	JN560258	JN560538	JN560538	JN560444	JN560619	JN560360	JN560685

Species	DNA source	Geographic origin of the sequenced material	<i>ITS</i> spacer	<i>trnL</i> intron	<i>trnL-F</i> spacer	<i>rpl20-rps12</i> spacer	<i>trnS-G</i> spacer	<i>psbA-trnH</i> spacer	<i>rbcL</i> gene
<i>Sicyos pachycarpus</i> Hook. & Arn.	A.K. Meebold 8477 (M)	Hawaii: Hawai'i	JN560259	EU436371	JN560539	JN560445		JN560361	
<i>Sicyos pachycarpus</i> Hook. & Arn.	I.R.H. Telford 10244 (CANB)	Hawaii: Maui	JN560260	JN560540	JN560540	JN560446	JN560620	JN560362	JN560686
<i>Sicyos pachycarpus</i> Hook. & Arn.	W.L. Wagner et al. 5807 (US)	Hawaii: Maui	JN560261	JN560541	JN560541	JN560447		JN560363	
<i>Sicyos palmatilobus</i> Cogn. ( <i>Anomalosicyos palmatilobus</i> (Cogn.) Gentry)	R. Spruce 6328 (K)	Ecuador	JN560262	JN560542		JN560448			
<i>Sicyos parviflorus</i> Willd.	I. Rodriguez et al. 234 (MEXU)	Mexico: México	JN560263	JN560543	JN560543	JN560449	JN560621	JN560364	JN560687
<i>Sicyos peninsularis</i> Brandegee	I. Rodriguez et al. 243 (MEXU)	Mexico: Nayarit	JN560264	JN560544	JN560544	JN560450	JN560622	JN560365	JN560688
<i>Sicyos polyacanthus</i> Cogn.	M.E. Múlgura de Romero et al. 1745 (NY)	Argentina: Misiones	JN560265	JN560545	JN560545	JN560451		JN560366	
<i>Sicyos polyacanthus</i> Cogn.	M. Nee et al. 50783 (NY)	Argentina: Jujuy		JN560546	JN560546	JN560452	JN560623	JN560367	
<i>Sicyos quinquelobatus</i> Cogn. ( <i>Anomalosicyos quinquelobatus</i> (Cogn.) Gentry)	M.M. Silva-Castro et al. 1021 (NY)	Brazil: Rio de Janeiro	JN560266	JN560547	JN560547	JN560453	JN560624	JN560368	
<i>Sicyos sertuliferus</i> Cogn.	J.V.A. Dieterle 4153 (MEXU)	Mexico: Jalisco	JN560267	JN560548	JN560548	JN560454	JN560625	JN560369	JN560689
<i>Sicyos sertuliferus</i> Cogn.	R. McVaugh 25200 (MEXU)	Mexico: Jalisco				JN560455	JN560626	JN560370	JN560690
<i>Sicyos triqueter</i> Ser. ( <i>Sechiopsis triqueter</i> (Ser.) Naudin)	J.V.A. Dieterle 4239 (MEXU)	Mexico: Jalisco	JN560205	JN560482	JN560482	JN560395	JN560577	JN560305	JN560646
<i>Sicyos undara</i> I.Telford & P.Sebastian sp. nov. ined.	D. Hansman s.n. CANB 9407 (CANB)	Australia: QLD	JN560268	JN560549	JN560549	JN560456	JN560627	JN560371	JN560691
<i>Sicyos undara</i> I.Telford & P.Sebastian sp. nov. ined.	I.R.H. Telford & P. Sebastian 13317 (NE)	Australia: QLD	JN560269	JN560550	JN560550	JN560457	JN560628	JN560372	JN560692
<i>Sicyos villosus</i> Hook.f.	C. Darwin s.n. (CGE)	Galapagos: Floreana	JN560270	JN560551	JN560551	JN560458			

<i>Sicyos waimanaloensis</i> H.St.John	S. Perlman & M. Burt 17510 (US)	Hawaii: Molokai	JN560271	JN560552	JN560552	JN560459	JN560629	JN560373	JN560693
<i>Sicyos warmingii</i> Cogn.	A. Krapovickas & G. Seijo 47831 (HUH)	Argentina: Salta	<i>JN560273</i>	<i>JN560554</i>	<i>JN560554</i>			<i>JN560374</i>	JN560694
<i>Sicyos warmingii</i> Cogn.	A. Krapovickas et al. 28341 (MO)	Argentina: Salta	<i>JN560272</i>	<i>JN560553</i>	<i>JN560553</i>				
<i>Sicyos warmingii</i> Cogn.	M. Nee et al. 52993 (NY)	Bolivia: Tarija	JN560274	JN560555	JN560555	JN560460	JN560630	JN560375	
<i>Sicyos weberbaueri</i> Harms	M. Weigend et al. 8534 (M)	Peru: Lambayeque	JN560275	JN560556	JN560556	JN560461	JN560631	JN560376	JN560695
<i>Sicyosperma gracile</i> A.Gray	M. Fishbein et al. 2565 (MO)	Mexico: Sonora	JN560276	DQ536867	DQ536867	DQ536595	JN560632	JN560377	DQ535772
<i>Sicyosperma gracile</i> A.Gray	V.W. Steinmann 961 (NY)	Mexico: Sonora		<i>JN560557</i>	<i>JN560557</i>	<i>JN560462</i>	<i>JN560633</i>	<i>JN560378</i>	<i>JN560696</i>
<i>Trichosanthes ovigera</i> Bl.	H. Takahashi 20711 (GIFU)	Japan		DQ536875	DQ536875	DQ536604			DQ535856



## CHAPTER 5

### *SICYOS* (CUCURBITACEAE) IN AUSTRALIA, NEW ZEALAND AND THE ISLANDS OF THE SOUTH-WESTERN PACIFIC

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*Australian Systematic Botany*, in review



## Abstract

Morphometric and molecular data reveal the existence of two new species of *Sicyos* in the south-western Pacific that had previously been confused with *S. australis* Endl. We here describe and illustrate these species, *S. undara* I.Telford & P.Sebastian and *S. mawhai* I.Telford & P.Sebastian, and emend the circumscription of *S. australis* to account for the removal of the extraneous elements. Distributions of the species, which are known from 10-14 collections, are mapped and habitat and conservation status noted. We also provide a key to the species of *Sicyos* in Australia and the south-western Pacific.

## Introduction

Phylogenetic reconstruction of Cucurbitaceae based on molecular data has revealed several unexpected relationships for the Australian genera and species (Kocyan *et al.*, 2007). For example, the monotypic genus *Nothoalsomitra* I.Telford, endemic to south-eastern Queensland (Telford, 1982), turned out to be sister to a large clade comprising the expanded Neotropical tribe Sicyoeae, which now includes the former Trichosantheae and the Luffineae of Benincaseae (Schaefer & Renner, 2011). *Sicyos*, the tribe's name-giving genus with 75 species (if segregate genera are included), has a center of diversity in the Neotropics but is also disjunctly distributed across the Pacific. A molecular phylogenetic study focusing on the biogeography of *Sicyos* showed that this distribution is the result of four trans-Pacific long-distance dispersals. In the Hawaiian Islands, *Sicyos* has undergone a remarkably large radiation with 14 species from a single dispersal event dated to only about two million years ago (Wagner *et al.*, 1990; Sebastian *et al.*, in review) while the second Pacific archipelago reached by *Sicyos*, the Galápagos Islands, has two endemic species that are the result of two arrivals about 4.5 and 1 million years ago. In the south-western Pacific region, *Sicyos* has been collected in eastern Australia, Tasmania, New Zealand, and smaller islands associated to these major landmasses as well as Lord Howe Island, Norfolk Island, and the Kermadec Islands.

Prior to the treatment of the Cucurbitaceae for the “Flora of Australia” (Telford, 1982), floras of this region considered *Sicyos* there to belong to a single species (Bentham, 1866; Bailey, 1900; Allan, 1961; Beadle *et al.*, 1963; Curtis, 1963, Willis, 1972; Beadle, 1976). Telford also recognized a single species, *S. australis* Endl., but indicated that the material was heterogeneous and in need of revision. The two names available for the Australian material, *S. australis* (Endlicher, 1833) and *S. fretensis* Hook.f. (Hooker, 1847), were used in K and BM, but had been little applied in Australian herbaria. Strangely, Hooker

(1860) later subsumed his Australian species, together with New Zealand material, into *S. angulatus* L. from the eastern United States, and this was followed by subsequent Flora writers cited above. Ferdinand von Mueller (1825 - 1896) annotated several sheets held in MEL and collected in Gippsland, Victoria, as *S. rivularis*, but had never validated the name. Based on more abundant material, Allan (1961) noted that New Zealand collections needed critical comparison with overseas *S. angulatus*, suggesting he had doubts about their conspecificity, and more recently, Brandon *et al.* (2004) recognised the New Zealand material as an endemic species, *S. aff. australis*, noting that *S. australis sensu stricto* also occurred on the North Island. Collections from Undara Lava Tubes in northern Queensland that became available after the *Flora of Australia* treatment also appeared to represent a new species, smaller in floral and fruit morphology than typical *S. australis*. A phenetic analysis of species boundaries was therefore conducted in 1997, and the results indicated two new species. Now, fourteen years later, a molecular phylogeny that includes material of most of the 75 species of *Sicyos* (Sebastian *et al.*, in review) confirmed the presence of three genetically distinct species of *Sicyos* in Australia and New Zealand. Here, we describe the two new species and provide an emended circumscription of *S. australis sensu stricto*.

## Materials and Methods

### Phenetic analysis

We here use a morphological species concept based on suites of co-varying attributes (Stuessy, 1990), and we tested the delimitation of our new species using graphical and statistical analysis of morphological data sets (Sneath & Sokal, 1973). The morphological dataset was derived from herbarium specimens held at AK, BM, BRI, CANB, CHR, HO, JCU, K, MEL, NE, NSW and W. Each organisational taxonomic unit (OTU) consisted of a single gathering, whether mounted on a single sheet or multiple sheets. Thirty-four OTUs were selected to cover the range of the genus in Australia and New Zealand (Table 1), and the characters used are listed in Table 2. States were assigned using dried herbarium specimens for inflorescence, fruit and seed characters, and rehydrated material for floral characters (Appendix 1); vegetative characters, such as leaf shape and pubescence, were avoided because of their well-known plasticity in the family.

The data matrix was analysed using the pattern analysis software package PATN version 3.03 (Belbin & Collins, 2006), with all characters given equal weight and Gower Metric used for the association measure. Two options of PATN (Belbin, 1990a, b) were used to define population groupings: cluster analysis, employing the flexible unweighted pair

group arithmetic averaging (UPGMA), presented graphically as a dendrogram, and ordination analysis, using semi-strong hybrid multidimensional scaling (SSH MDS) in a three-dimensional scatter plot.

**Table 1.** OTU numbers, codes used in analyses and vouchers for phenetic analysis. Voucher data presents collector's name, number, and herbarium where housed. Herbarium accession number is used when collector's number is absent. Herbarium codes follow Index Herbariorum: <http://sweetgum.nybg.org/ih/>.

OTU	Code	Locality	Collector
1	NZ1	Cuvier Is., New Zealand	R. Moynihan AK119648 (AK)
2	NZ2	Hen Is., New Zealand	D. Merton CHR183501(CHR)
3	NZ3	Little Barrier Is., New Zealand	W.R. Sykes 147/85 (CHR)
4	NZ4	Auckland, New Zealand	J.J. Bruhl 2915 (NE)
5	NZ5	Coromandel Is., New Zealand	A.E. Esler 3584 (CHR)
6	NZ6	Tahuna Rd, Te Teko, New Zealand	W. Stahel CHR566019 (CHR)
7	NZ7	White Pine Bush, New Zealand	M. Steverinson & P. Cashmore CHR551181(CHR)
8	NZ8	Poor Knights Is., New Zealand	G.N. Park CHR276071 (CHR)
9	NZ9	Pukaraka, New Zealand	K. Riddell AK238904 (AK)
10	NZ10	Whenuakite, New Zealand	C. Wallace AK199785 (AK)
11	NZ11	Three Kings Is., New Zealand	A.E. Wright 5266 (AK)
12	NZ12	Three Kings Is., New Zealand	A.E. Wright 6061 (AK)
13	KI1	Kermadec Is.	T.F. Cheeseman MEL591390 (MEL)
14	KI2	Kermadec Is.	C.J. West CHR518213 (CHR)
15	KI3	Kermadec Is.	W. Sykes 1523/K (CHR)
16	NI1	Norfolk Is.	W. Robinson (K)
17	NI2	Norfolk Is.	F. Bauer 110 (W)
18	LH1	Lord Howe Is.	leg. ign. MEL593267-8 (MEL)
19	LH2	Lord Howe Is.	J. Fullagar 118 (MEL)
20	LH3	Lord Howe Is.	leg. ign. (K)
21	TS1	Sisters Is., Tasmania	J. Whinray 57 (CANB)
22	TS2	Sisters Is., Tasmania	J. Whinray 492 (CANB)
23	VG1	Buchan River, Victoria	F. Mueller MEL593278(MEL)
24	VG2	Tambo River, Victoria	F. Mueller MEL593283 (MEL)
25	NS1	Tuross River, New South Wales	J. Whaite 478 (NE)
26	NS2	Cambewarra Mtn, New South Wales	I.R. Telford 11793 (CANB)
27	NS3	Nymboida River, New South Wales	J.J. Bruhl 2102 (NE)
28	QL1	Killarney, Queensland	I.R. Telford 12909 (NE)
29	QL2	Isla Gorge, Queensland	P. Sharpe 660 (BRI)
30	QL3	Eungella, Queensland	N. Byrnes 3699 (BRI)
31	QL4	Fanning River Caves, Queensland	B. Jackes S8643 (JCU)
32	QL5	Wind Tunnel, Undara, Queensland	I.R. Telford 13319 & P. Sebastian (NE)
33	QL6	Pinwill Cave, Undara, Queensland	V.J. Neldner 2780 (BRI)
34	QL7	Pinwill Cave, Undara, Queensland	I.R. Telford 13317 & P. Sebastian (NE)

**Table 2.** Morphological characters used for ordination and cluster analysis of *Sicyos* in the south-western Pacific

No.	Character
Female inflorescence	
1	Peduncle length (mm)
2	Flowers per inflorescence
3	Corolla diameter (mm)
Fruit	
4	Fruit length (mm)
5	Fruit diameter (mm)
6	Aculei length (mm)
7	Aculei per fruit
8	Seed length (mm)
Male inflorescence	
9	Inflorescence length (mm)
10	Peduncle length (mm)
11	Flower number per inflorescence
12	Pedicle length (mm)
13	Corolla diameter (mm)
14	Staminal column length (mm)
15	Staminal head diameter (mm)

**Table 3.** Correlation of characters values with correlation of attributes for SSH MDS ordination of *Sicyos* in the south-western Pacific

Character	X	Y	Z	r-squared
5	0.798	0.355	-0.488	0.923
8	0.298	0.666	-0.684	0.918
7	0.255	0.377	-0.89	0.821
15	-0.176	0.31	-0.934	0.81
13	0.662	0.616	-0.428	0.796
4	0.467	0.623	-0.627	0.782
12	0.788	0.616	-0.019	0.734
9	0.557	-0.171	-0.812	0.71
3	0.8	0.009	-0.601	0.707
14	-0.628	0.123	-0.769	0.625
6	-0.649	-0.186	-0.738	0.62
10	0.292	-0.457	-0.84	0.534
11	0.966	-0.023	-0.257	0.508
1	0.778	0.187	0.6	0.446
2	0.463	0.797	-0.388	0.265

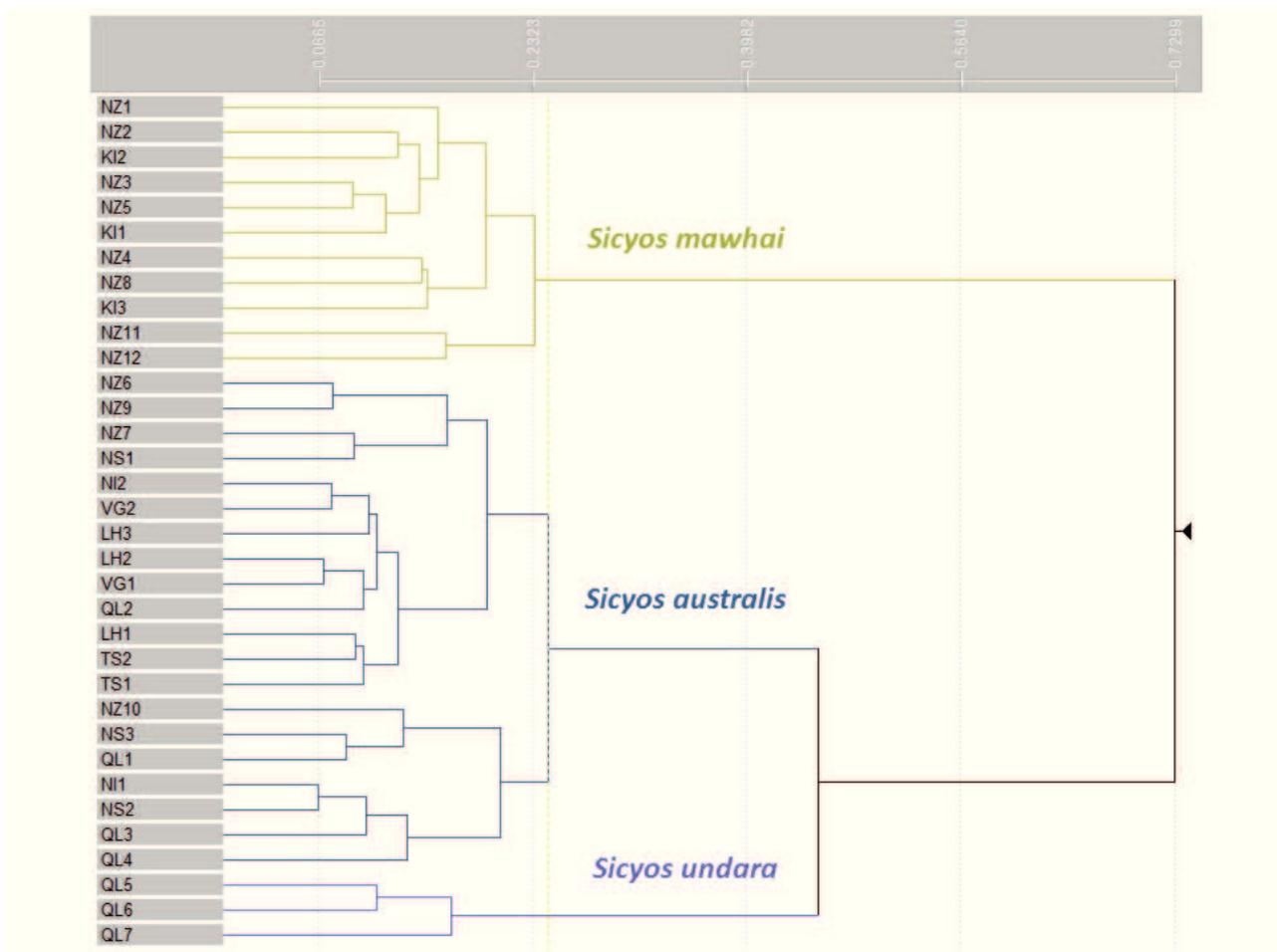
## Molecular analysis

For the molecular phylogeny, we used the matrix of Sebastian *et al.* (in review), which includes 112 accessions representing 87 species of Sicyoeae, including the type species of all relevant generic names. The matrix comprised six chloroplast regions, the *trnL* intron, the *trnL-F*, *rpl20-rps12*, *trnS-G*, and *psbA-trnH* intergenic spacers, and the *rbcL* gene as well as the nuclear ribosomal DNA internal transcribed spacers ITS1 and ITS2, with the intervening 5.8 S gene, for a length of 5399 aligned positions (4527 chloroplast and 872 nuclear). GenBank accession numbers and voucher information are listed in Table S1 of Sebastian *et al.* (in review). DNA-extraction, polymerase chain reactions, sequencing, and data preparation were carried out using standard approaches (Sebastian *et al.*, 2010; Sebastian *et al.*, in review). Maximum likelihood (ML) analyses and ML bootstrap searches using 500 replicates were carried out using RAxML version 7.2.8 (Stamatakis, 2006), relying on the GTR +  $\Gamma$  model, with model parameters estimated over the duration of specified runs. Molecular clock analyses and ancestral area reconstruction were carried out simultaneously using Bayesian time estimation and a relaxed clock uncorrelated-rates model, fossil calibration, and a continuous-time Markov chain (CTMC) phylogeographic model with stochastic search variable selection as implemented in the BEAST package version 1.6.1 (Drummond & Rambaut, 2007). Complete methods are given in Sebastian *et al.* (in review).

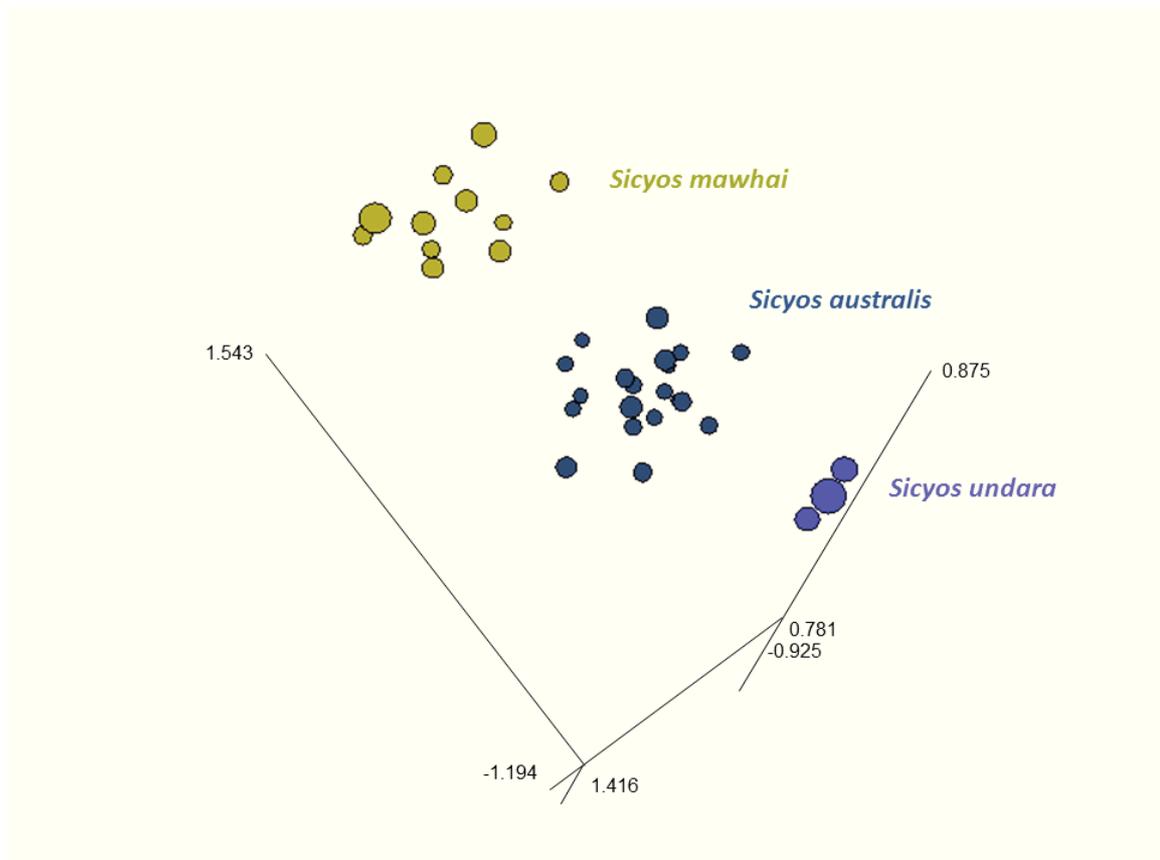
## Results

### Phenetic analysis

Phenetic patterns from UPGMA clustering (Fig. 1) and SSH MDS ordination (Fig. 2) recover the same three groups. Table 3 shows the correlation of characters with ordination patterns: fruit and seed attributes contributed most to phenetic differences. The results indicate that two species should be recognised in Australia, the widespread *S. australis* (OTUs 17 - 32) and the putative new species from Undara (OTUs 33 - 35), its name validated below. *Sicyos fretensis* (OTUs 22, 23) and Mueller's unpublished *S. rivularis* (OTUs 24, 25) are grouped within *S. australis*, the former thus rightly placed in synonymy under the latter name. New Zealand material groups into two species, the putative new species (OTUs 1 - 5, 8, 9, 11 - 16), named below, and those (OTUs 6, 7, 10) grouping with *S. australis*, confirming their placement by Brandon *et al.* (2004). This interpretation is corroborated by the molecular phylogenetic analysis (below), with the exception of *S. australis* in New Zealand, where no sample has yet been included.



**Fig. 1.** Phenogram based on Gower association and flexible UPGMA of the OTUs of *Sicyos* in the south-western Pacific. See Table 1 for OTU coding and vouchers.



**Fig. 2.** 3D-ordination of SSH MDS for OTUs of *Sicyos* in the south-western Pacific. Stress = 0.0847.

### Molecular analysis

The three Australian and New Zealand species that are the focus of this study are derived from a common ancestor (Fig. 3) and are sister to a clade of species from the south-western United States, Mexico, and Bolivia. They diverged from their North American ancestor 5.6 - 1.7 million years ago (Fig. 3, inset). Although with low statistical support, both the maximum likelihood (Fig. 3) and Bayesian analyses (not shown here, but see Figs. 3 and S1 of Sebastian *et al.*, in review) recovered the newly described Australian species *S. undara* as sister to the other two, diverging at 3.6 - 0.5 million years ago. *Sicyos australis* and the New Zealand endemic *S. mawhai* split from each other at 1.6 - 0.1 million years ago.



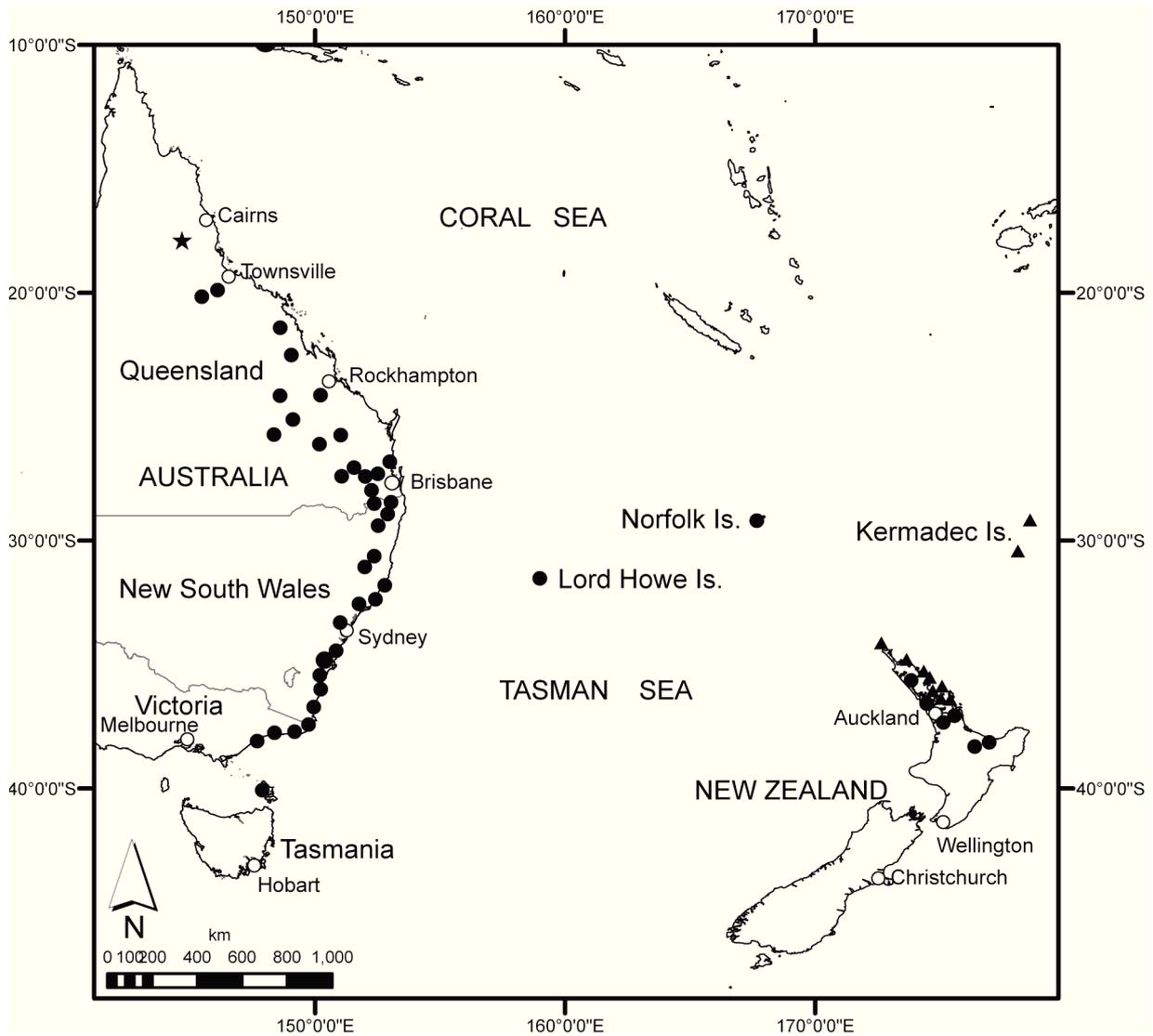
**Fig. 3.** Maximum likelihood phylogram for 87 taxa of Sicyoeae based on combined sequences from chloroplast and nuclear data (5399 aligned nucleotides), analysed under the GTR +  $\Gamma$  substitution model. The tree is rooted on *Nothoalsomitra*. Likelihood bootstrap values  $\geq 75\%$  are given at the nodes; geographic origin of the sequenced specimens follows the species name, and stars indicate generic type species. *Inset*: Node ages, obtained under a Bayesian relaxed clock with log-normally distributed rates, are given at nodes of interest. Letters at internal nodes give the most likely areas of origin (posterior probability  $\geq 0.91$ ) in North America (A), South America (B), or the south-western Pacific (C), as inferred by ancestral area reconstruction under a continuous-time Markov chain phylogeographic model.

## Discussion

*Sicyos mawhai* represents the first endemic species of the Cucurbitaceae family from New Zealand known to date. While the new species from Australia and New Zealand described here are only found in a few, possibly endangered populations in restricted areas, *S. australis* is rather widespread, especially in Australia, where it occurs in various habitats (Fig. 4 for a distribution map of all three species), similar to *S. angulatus*, which is widespread in many disturbed habitats of North America and can even be invasive in the Old World. In New Zealand, populations of *S. australis* are threatened by common cucurbit diseases, such as *Cucumber mosaic virus* (CMV) or *Zucchini yellow mosaic virus* (ZYMV) (Delmiglio & Pearson, 2006), which may also be responsible for the decline of the two *Sicyos* species on the Galápagos archipelago (Sebastian *et al.* 2010b). Australian populations, by contrast, appear healthy, with no obvious decline through disease (IRT pers. obs.).

Two dispersal events from Australia to New Zealand are the most plausible interpretation of the phylogeny, the first giving rise to *S. mawhai*, the second, probably more recently by *S. australis*, perhaps via the island stepping stones of Norfolk or Lord Howe Islands. Cucurbitaceae in general are successful transoceanic dispersers (Schaefer *et al.*, 2009), and the trans-Pacific range disjunctions of *Sicyos* are discussed in detail in Sebastian *et al.* (in review). Long-distance dispersals as in our focal clade (Fig. 3) across the *c.* 2000 km of Tasman Sea from Australia to New Zealand are rather common with numerous plant genera disjunctly distributed in this area (Perrie *et al.*, 2003; Chandler *et al.*, 2007; Tay *et al.* 2010; Prebble *et al.*, 2011).

Like their closest relatives from North and South America, the three Australian and New Zealand species bear retrorsely barbed spines on their small, one-seeded fruits. Spines were lost several times across the phylogeny (see Fig. S3 of Sebastian *et al.*, in review), but the ability to disperse over long distances seems correlated with the presence of barbed spines on the fruits of the common ancestor. The spiny fruits of *Sicyos* are likely to adhere to birds' plumage and thus be transported across their migration routes. Both *S. australis* and *S. mawhai* can be found in coastal vegetation, and they are likely to come in contact with sea-birds like petrels, shearwaters and albatrosses that regularly travel between the southern Pacific landmasses and are a potential group of dispersal agents (McGlone *et al.*, 2001; Winkworth *et al.*, 2002).



**Fig. 4.** Distribution of *Sicyos* in the south-western Pacific. ● *S. australis*; ▲ *S. mawhai*; ★ *S. undara*.

## Taxonomy

*Sicyos australis* Endl., Prodrromus Flora Norfolkicae 67 (1833)

**Type:** NORFOLK ISLAND, western mountain, *F. Bauer 110*; holo: W.

*S. fretensis* Hook.f., *Hooker's London Journal of Botany* 6: 473 (1847).

Type: Sisters' Island, E coast of Flinders Island, *R. Gunn* [Oct., 1844, *J. Milligan* 563]; holo: K; iso: BM, HO, K, MEL.

*S. angulatus auct. non* L.; Benth., *Flora Australiensis* 3: 322 (1866); Bailey, *Queensland Flora* 701 (1900); Beadle *et al.*, *Flora of the Sydney Region* 199 (1963); Curtis, *The Student's Flora of Tasmania* 237 (1963); Willis, 2: 264 (1972); Beadle, *Students Flora of North-eastern New South Wales* 271 (1976).

Herbaceous climber; stems annual, to 10 m long, 5 mm diam., sparsely hirsute with simple multicellular hairs and glandular hairs, glabrescent. Tendrils 3 – 5-branched. Leaves: petiole 10 - 65 mm long; lamina ovate or broadly ovate in outline, 35 - 195 mm long, 45 - 200 mm wide, cordate with the basal sinus broad, the lobes not overlapping, acuminate, shallowly to deeply palmately 5- or 7-lobed, the lobes broadly triangular, acute or acuminate, margins dentate with apiculate teeth, sparsely scaberulous adaxially and abaxially with simple hairs, more densely and coarsely along veins. Male inflorescence an 8–19-flowered raceme 25 - 110(-155) mm long; peduncle 15(-125) mm long, sparsely scaberulous; rhachis glandular hairy. Male flowers: pedicels 3 - 11 mm long; hypanthium broadly campanulate, 2.4 - 2.7 mm diam.; calyx lobes linear, 0.4 - 0.7 mm long; corolla rotate, 4.5 - 7.8 mm diam., mostly glabrous abaxially, the lobe apices puberulous, glabrous adaxially, white, 5-lobed, the lobes broadly triangular-ovate, obtuse; 2 - 2.6 mm long; disc *c.* 1.2 mm diam.; staminal column 1.5 - 1.8 mm long; staminal head 1.7 - 2.2 mm diam. Female inflorescence an 8 - 11-flowered head; peduncle 8 - 23(-33) mm long. Female flowers: subsessile; ovary ovate, attenuate, *c.* 3 mm long, 1.4 mm diam., minutely and densely echinulate with barbed aculei; hypanthium above the constriction broadly campanulate, *c.* 1.5 mm diam., minutely glandular hairy surrounding the disc; calyx lobes linear, 0.4 - 0.7 mm long; corolla 2.5 - 3.5 mm diam., mostly glabrous abaxially, the lobe apices puberulous, glabrous adaxially, white, 5-lobed, the lobes triangular-ovate, obtuse, *c.* 1.6 mm long; disc *c.* 1 mm diam.; style *c.* 1.4 mm long; stigma 2-lobed, the lobes *c.* 0.3 mm long, recurved. Fruit ovate, rarely fusiform, 6.4 - 9.5 mm

long, 2.8 - 4 mm diam., apically attenuate; densely echinate, aculei dense, 1.6 - 3.6 mm long, retrorsely barbed. Seeds ellipsoidal, 4 - 5.5 mm long, 2.8 - 3.4 mm wide, brown.

#### *Distribution*

*Sicyos australis* occurs in eastern Australia from Fanning River, c. 60 km SW of Townsville, Queensland, south to Orbost, Victoria, inland to the Great Dividing Range, and on Sisters' Island, off Flinders Island, Tasmania. The first record in New Zealand was collected at Thames, North Island, by T.F. Cheeseman of the Auckland Museum, the collection date not recorded, but probably in the 1880s. There are several recent collections from the Bay of Plenty area, North Island. Previously on Lord Howe and Norfolk Islands, the species has not been collected for more than a century and is presumed extinct there (Green, 1994).

#### *Habitat and ecology*

*Sicyos australis* grows in dry rainforest (deciduous vine thicket) with *Brachychiton australis* on basalt or limestone in northern Queensland, and with *Brachychiton rupestris* and *Acacia harpophylla* on cracking clay derived from basalt in central Queensland. In coastal and near-coastal montane habitats from Eungella, Queensland, south to Gippsland, Victoria, *S. australis* grows in wetter eucalypt forest or on rainforest margins, in disturbed sites in closed forest, often in sheltered sites in gorges and on a wide variety of substrates.

In New Zealand, *S. australis* has been collected from weedy, disturbed areas, suggesting an invasive recent arrival. However, the 19<sup>th</sup> century collection by Cheeseman cited above indicates that the species had become established in the North Island by the 1880s.

#### *Phenology*

Flowers and fruits November to July.

#### *Conservation status*

Widespread and common throughout its present range in mainland Australia, *S. australis* is not considered at risk; it is also conserved in many national parks. In New Zealand, the species is rated "Nationally critical" by the New Zealand Plant Conservation Network, Waikato Conservancy (Brandon *et al.*, 2004).

*Notes*

The protologue of *S. fretensis* attributes the type collection to Ronald Gunn. Gunn's private herbarium included specimens collected by others, sometimes with label data transcribed without collector, and these were often assumed to have been collected by Gunn himself (Buchanan, 1988).

*Illustrations*

D.L. Jones & B. Gray, Australian Climbing Plants fig. 227 (1977); N.G. Walsh & T.J. Entwisle (eds), Flora of Victoria 3: 384 fig. 79h (1996).

*Additional specimens examined (selection)*

NORFOLK ISLAND: 1898, *W. Robinson s.n.* (K, NSW). NEW ZEALAND: North Island: side of Tahuna Road, Te Teko (between Rotorua and Whakatane), *W. Stahel CHR566019* (CHR); White Pine Bush near Awakeri, maize paddock at end of Ernest Road, *M. Steverinson & P.B. Cashmore NZFRI26280* (AK, CHR, NZFRI). AUSTRALIA: Queensland: North Kennedy District: Fanning River, N of Mingela, *B.R. Jackes S8643* (JCU, NSW); South Kennedy District: Eungella, *N. Byrnes 3699 & J. Clarkson* (BRI); Leichhardt District: Ka Ka Mundi section, Carnarvon National Park, on fire trail from Salvator Rosa, *M.B. Thomas 3781 & C. Elmes* (BRI); Darling Downs District: Great Dividing Range, c. 15 km ENE of Killarney, *I.R. Telford 12909, J.J. Bruhl & L.M. Copeland* (CANB, M, NE). New South Wales: Lord Howe Island: no precise loc., *J. Fullagar 118* (MEL); North Coast: Nymboi-Binderay National Park, Platypus Flat, *J.J. Bruhl 2102 & F.C. Quinn* (BRI, CANB, L, MO, NE); Central Coast: Cambewarra Mountain, Beaumont, *I.R. Telford 11793* (CANB, NSW); South Coast: Morton National Park, Sentrybox Canyon, *I.R. Telford 10805* (CANB, K, NSW); Tuross River, *J. Whaite 478* (NE). Victoria: Buchan River, *F. Mueller s.n.* MEL 593278 (MEL). Tasmania: Sisters' Island, *J. Whinray 500* (CANB, MEL).

***Sicyos undara*** I.Telford & P.Sebastian **sp. nov.**

Ab affini *Sicyo australi* inflorescentiis foemineis paucifloris longius et tenuius pedunculatis nec non fructibus minoribus parcius et minutius aculeatis differt.

**Type:** AUSTRALIA: Queensland: Cook District: Undara Volcanic National Park, 50 m W of Pinwill Cave entrance, 27 Mar. 2009, *I.R. Telford 13317 & P. Sebastian*; holo: BRI; iso: BISH, CANB, CHR, CNS, K, M, MO, NE, US (Fig. 5: isotype NE).

Herbaceous climber; stems annual, to 5 m long, to 2.5 mm diam., sparsely hirsute with simple multicellular hairs and glandular hairs, densely so at nodes, glabrescent. Tendrils 3- or 4-branched. Leaves: petiole 10 - 35 mm long, sparsely pilose multicellular and glandular hairs; lamina subtriangular or broadly ovate in outline, 20 - 117 mm long, 20 - 94 mm wide, cordate with the basal sinus broad, the lobes not overlapping, acuminate, palmately shallowly 3- or 5-lobed, the lobes triangular, acuminate, margins dentate with apiculate teeth, hispid on both surfaces, more coarsely so on veins abaxially. Male inflorescence a 7 - 25-flowered raceme 35 - 155(-180) mm long; peduncle 24 - 108 mm long, glabrous; rhachis glandular hairy. Male flowers: pedicel 5 - 21 mm long; hypanthium broadly campanulate, *c.* 1.8 mm diam.; calyx lobes linear, *c.* 0.7 mm long; corolla rotate, 3 - 5.7 mm diam., abaxially and adaxially glabrous, the margins glandular hairy, greenish white, 5-lobed, the lobes triangular; 1 - 1.8 mm long; disc *c.* 1 mm diam.; staminal column 0.9 - 1.1 mm long; staminal head 0.7 - 0.8 mm diam.. Female inflorescence a 4-8-flowered head; peduncle (20-)32 - 74 mm long. Female flowers: subsessile; ovary ovate, *c.* 1.2 mm long, *c.* 0.7 mm diam., echinulate with barbed aculei; hypanthium above the constriction broadly campanulate, *c.* 1.3 mm diam.; calyx lobes linear, *c.* 0.7 mm long; corolla 2.6 - 3 mm diam., glabrous abaxially and adaxially, the margins minutely glandular papillose, greenish white, 5-lobed, lobes triangular, *c.* 1 mm long; disc *c.* 0.5 mm diam; style *c.* 1.5 mm long; stigma 2-branched, the branches recurved. Fruit ovate or subglobose, 4 - 5.8 mm long, 2.2 - 2.6 mm diam., apically rounded or obtuse; aculei scattered, 1.4 - 2.8 mm long, retrorsely barbed. Seeds  $\pm$ ovate, *c.* 3.5 mm long, 2 mm wide, cream. (Fig. 5)

*Distribution*

*Sicyos undara* is known only from the Undara Lava Tubes, east of Mount Surprise, Queensland.

*Habitat and ecology*

*Sicyos undara* grows in skeletal clay loam in boulder gullies formed by collapsed lava tubes. The vegetation is deciduous vine thickets (dry rain forest) dominated by *Brachychiton australis* and *Melia azederach* with *Pittosporum spinescens*. Undara Lava Tubes form part of the Cainozoic McBride Volcanic Province (Griffin & McDougall, 1975), the basalts of which provide habitat for several narrowly endemic plant species including *Aurantiarpa edentata* and *Ipomoea saintronanensis*.

*Phenology*

Flowers and fruits March to April.

*Conservation status*

*Sicyos undara* is known from two subpopulations at Undara, one with a single plant observed in March 2009, the other with two. A survey is necessary to establish the range of the species, which would be considered DD (data deficient) when using the IUCN categories (2001; IUCN, 2008) or threatened under the *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth). Too frequent wildfires would constitute a major threat. The species is conserved in Undara Volcanic National Park.

*Etymology*

Named for the only known locality of the species, from the local aboriginal “a long way”.

*Additional specimens examined*

QUEENSLAND: Cook District: Yaramula Station [now Undara Volcanic National Park], adjacent to Pinwill Cave, 80 km SW of Mount Garnet, *V.J. Neldner* 2780 (BRI, CANB, CNS); Undara, Wind Tunnel, 23 Mar. 1994, *D. Hansman s.n.* (CANB); Undara Volcanic National Park, Wind Tunnel complex, *I.R. Telford* 13319 & *P. Sebastian* (BRI, CANB, CNS, M, NE).



Fig. 5. Isotype of *Sicyos undara* I.Telford & P.Sebastian

***Sicyos mawhai*** I.Telford & P.Sebastian **sp. nov.**

Ab affini *Sicyo australi* inflorescentiis floribusque masculis majoribus, floribus foemineis majoribus in quoque capitulo numerosioribus nec non fructuum aculeis longioribus discrepat.

**Type:** NEW ZEALAND: North Island: Cuvier Island, large patch scrambling over bracken on ridgetop, West Point, 25 May 1980, *A.E. Wright* 3583; holo: AK; iso: A, AD, AKU, BISH, CM, LTU, NA, WELT; all n.v. except AK, AD.

*S. angulatus auct. non L.:* Allan, Flora of New Zealand 319 (1961).

Herbaceous climber; stems annual, to 10 m long, to 2.5 mm diam., sparsely hirsute with simple multicellular celled hairs and glandular hairs, glabrescent. Tendrils 3 - 5-branched. Leaves: petiole 25 - 75(-135) mm long; lamina broadly ovate in outline, 45 - 135 mm long, 60 - 165 mm wide, cordate with the basal sinus  $\pm$  closed by overlapping lobes, acuminate, shallowly palmately 5- or 7-lobed, the lobes rounded-triangular, margins coarsely dentate with apiculate teeth. Male inflorescence a 14 - 35-flowered raceme (45-)70 - 85(-205) mm long; peduncle (35-)55 - 90(-125) mm long. Male flowers: pedicels (3.5-)6 - 12(-27) mm long; hypanthium broadly campanulate, 3.8 - 4.2 mm diam.; calyx lobes linear, *c.* 1.2 mm long; corolla rotate, (7-)10 - 13 mm diam., 5-lobed, the lobes rounded-triangular, 2 - 3.5 mm long; disc *c.* 2 mm diam.; staminal column 1.8 - 2.2 mm long; staminal head 1.9 - 2.4 mm diam. Female inflorescence a (6-)9 - 14(-20)-flowered head; peduncle 12 - 35 mm long. Female flowers: subsessile; ovary ovate, 3.2 - 4 mm long, 2 - 2.6 diam., echinulate with barbed aculei; hypanthium above the constriction broadly campanulate, *c.* 2.4 mm diam., abaxially scabridulous; calyx lobes linear, *c.* 0.8 mm long; ; corolla 4 - 6.8 mm diam., glabrous abaxially and adaxially, glandular papillose on margins, white, 5-lobed; lobes triangular-ovate, 2 - 2.3 mm long; disc *c.* 1.3 mm diam.; style *c.* 1.8 mm long; stigma 2-branched, the branches recurved. Fruit ovate, 8 - 13 mm long, 4 - 5.8 mm diam., obtuse or subacute; aculei dense, (2.2-)3.6 - 6 mm long, retrorsely barbed, the fruit surface scabridulous with shorter hairs. Seeds  $\pm$  ellipsoidal, 5.6 - 6 mm long, 3.8 - 4.5 mm wide, brown. (Fig. 6)

*Distribution*

*Sicyos mawhai* occurs on New Zealand's North Island and adjacent islands from the Coromandel Peninsula northwards including Three Kings Islands and the Kermadec Islands.

*Habitat and ecology*

*Sicyos mawhai* grows in near-coastal sites, often on beach strands, in clays or loams on volcanic soils. Vegetation communities include shrubberies or forest margins with *Brachyglottis arborescens*, *Melicytus ramiflorus* and *Metrosideros excelsa* or in open, sometimes disturbed, areas with grasses and *Pteridium esculentum*.

*Phenology*

*Sicyos mawhai* flowers and fruits mainly December to May.

*Conservation status*

*Sicyos mawhai* is known from five sites on the mainland of North Island but is more common on adjacent islands and the Kermadec Islands. Plants are susceptible to *Cucumber mosaic virus* and *Zucchini yellow mosaic virus* (Delmiglio & Pearson, 2006) and this appears to weaken populations. Habitat loss by coastal development is another potential threat. The species is rated “in serious decline” by the New Zealand Plant Conservation Network, Waikato Conservancy as *S. aff. australis* (Brandon *et al.*, 2004).

*Notes*

*Sicyos mawhai* and *S. australis* occur almost sympatrically, but can readily be distinguished vegetatively; the former has leaves of a thicker texture, the lobes more rounded, with the basal pair sometimes overlapping across the sinus, as well as by the floral and fruit differences presented in the key below.

*Etymology*

Named for the Maori name of the plant, “useless,” in reference to the possible confusion of its seedlings with those of the cultivated bottle gourd, *Lagenaria siceraria* (Molina) Standl. (P. de Lange, pers. comm.).

*Illustration*

F.B. Sampson, Early New Zealand Botanical Art t. 28 (1985) as *Sicyos angulatus*.

*Additional specimens examined (selection)*

NEW ZEALAND: Kermadec Islands, Raoul Island orchard, *W.R. Sykes 188/K* (CHR, K); Raoul Island, Low Flat, *C.J. West s.n.* CHR 518213 (CHR); Kermadec Islands, Macauley Island, Mount Haszard, *G. Taylor 13* (CHR). Three Kings Islands, West Island, *A.E. Wright 6061* (AK); Great Island, *J.S. Edwards s.n.* AK 44826 (AK); Poor Knights Islands, Aorangi Island, Crater Bay, *G.N. Park CHR276071* (CHR); Hen and Chickens Islands, *R. Beever s.n.* AK106168 (AK); Little Barrier Island, near West Landing, *W.R. Sykes 147/85* (CHR); Fanal Island, *A.E. Wright 3146* (AK, HO, WELT); Auckland, *J.J. Bruhl 2915* (AK, CHR, M, NE); Coromandel Islands, Motukarikitahi, *A.E. Esler 3584* (CHR).



Fig. 6. Holotype of *Sicyos mawhai* I. Telford & P. Sebastian

## Key to *Sicyos* in the south-western Pacific

- Male flowers mostly > 10 mm diam. ....*S. mawhai*
- Male flowers mostly < 10 mm diam.
- Fruit > 6 mm long, attenuate; peduncle of female inflorescence mostly < 25 mm long  
 .....*S. australis*
- Fruit < 6 mm long, apically rounded; peduncle of female inflorescence mostly > 25 mm long  
 .....*S. undara*

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## References

- Allan, H.H. (1961) *Flora of New Zealand*. Government Printer, Wellington.
- Bailey, F.M. (1900) *The Queensland Flora*, Queensland Government, Brisbane
- Beadle, N.C.W. (1976) *Students flora of north eastern New South Wales. Part III. Angiosperms: Families 84-106*. Botany Department, University of New England, Armidale.
- Beadle, N.C.W., Carolin, R.C. & Evans, O.D. (1963) *Handbook of the Vascular Plants of the Sydney District and the Blue Mountains*. A.H. and A.W. Reed, Sydney.
- Belbin, L. (1990a) *PATN: A User's Guide*. Division of Wildlife and Ecology, C.S.I.R.O., Canberra.
- Belbin, L. (1990b) *PATN: A Technical Reference*. Division of Wildlife and Ecology, C.S.I.R.O., Canberra.
- Belbin, L. & Collins, A. (2006) *PATN. V.3.1*. Blatant Fabrications Pty Ltd, Brisbane.
- Bentham, G. (1866) *Flora Australiensis*. Lovell Reeve & Co., London.
- Brandon, A., de Lange, P. & Townsend, A. (2004) *Threatened Plants of the Waikato Conservancy*. Department of Conservation, Wellington.
- Buchanan, A.M. (1988) The Tasmanian collecting localities of Ronald Gunn and Joseph Milligan. *Tasmanian Herbarium Occasional Publication*, 1.
- Chandler, G.T., Plunkett, G.M., Pinney, S.M., Cayzer, L.W. & Gemmill, C.E.C. (2007) Molecular and morphological agreement in Pittosporaceae: phylogenetic analysis with

- nuclear ITS and plastid *trnL-tmF* sequence data. *Australian Systematic Botany*, **20**, 490-401.
- Curtis, W.M. (1963) *The students flora of Tasmania*. Government Printer, Hobart.
- Delmiglio, C. & Pearson, M.N. (2006) Effects and incidence of *Cucumber mosaic virus*, *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* in New Zealand's only native cucurbit, *Sicyos australis*. *Australasian Plant Pathology*, **35**, 29-35.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Endlicher, S.F.L. (1833) *Prodromus Flora Norfolkicae*. F. Beck, Vienna.
- Green, P.S. (1994) Oceanic Islands 1. *Flora of Australia* vol. 49 (ed. by A.J.G. Wilson), Australian Government Publishing Service, Canberra.
- Griffin, T.J. & McDougall, I. (1975) Geochronology of the Cainozoic McBride Volcanic Province, Northern Queensland. *Journal of the Geological Society of Australia*, **22**, 387-396.
- Hooker, J.D. (1847) Flora Tasmaniae spicelegium. *Hooker's London Journal of Botany*, **6**, 473-474.
- Hooker, J.D. (1860) *The Botany of the Antarctic Voyage Part 3: Flora Tasmaniae*. Lovell Reeve & Co., London.
- IUCN (2001) *Red listed categories and criteria: version 3.1*. IUCN, Gland, Switzerland.
- IUCN Standards and Petitions Working Group (2008) *Guidelines for using the IUCN Red List categories and criteria, Version 7.0*. Available at: <http://intranet.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf> (last accessed: August 2011)
- Jobson, P.C. (1996) Cucurbitaceae. *Flora of Victoria* (ed. by N.G. Walsh and T.J. Entwistle), Inkata Press, Melbourne.
- Jones, D.J. & Gray, B. (1977) *Australian climbing plants: with notes on their cultivation*. Reed, Sydney.
- Kocyan, A., Zhang, L.-B., Schaefer, H. & Renner, S.S. (2007) A multi-locus chloroplast phylogeny of the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553-577.
- McGlone, M.S., Duncan, R.P. & Heenan, P.B. (2001) Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography*, **28**, 199-216.
- Perrie, L.R., Brownsey, P.J., Lockhart P.J., Brown, E.A. & Large, M.F. (2003) Biogeography of temperate Australasian *Polystichum* ferns inferred from chloroplast sequence and AFLP. *Journal of Biogeography*, **30**, 1729-1736.
- Prebble, J.M., Cupido, C.N., Meudt, H.M. & Garnock-Jones, P.J. (2011) First phylogenetic and biogeographical study of the southern bluebells (*Wahlenbergia*, Campanulaceae). *Molecular Phylogenetics and Evolution*, **59**, 636-648.
- Sampson, F.B. (1985) Early New Zealand Botanical Art. Reed Methuen, Auckland.
- Schaefer, H. & Renner, S.S. (2011) Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon*, **60**, 122-138.
- Sebastian, P., Schaefer, H., Telford, I.R.H. & Renner, S.S. (2010) Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Science USA*, **107**, 14269-14273.
- Sebastian, P., Schaefer, H., Lira, R., Telford, I.R.H. & Renner, S.S. Radiation following long-distance dispersal: the contributions of time, opportunity, and diaspore morphology in *Sicyos* (Cucurbitaceae). In review.
- Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical Taxonomy: the principles and practice of numerical classification*. W.H. Freeman & Co., San Francisco.

- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analysis with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688-2690.
- Stuessy, T.F. (1990) *Plant taxonomy: the systematic evaluation of comparative data*. Columbia University Press, New York.
- Tay, M.L., Meudt, H.M., Garnock-Jones, P.J. & Ritchie, P.A. (2010) DNA sequences from three genomes reveal multiple long-distance dispersals and non-monophyly of sections in Australasian *Plantago* (Plantaginaceae). *Australian Systematic Botany*, **23**, 47-68.
- Telford, I.R.H. (1982) Cucurbitaceae. *Flora of Australia* vol 8. (ed. by A.S. George), pp. 158-198. Australian Government Publishing Service, Canberra.
- Wagner, W.L., Herbst, D.R. & Sohmer, S.H. (1990) *Manual of the flowering plants of Hawai'i*. University of Hawaii Press & Bishop Museum Press, Honolulu.
- Willis, J.H. (1972) *A Handbook to Plants in Victoria*. Melbourne University Press, Carlton.
- Winkworth, R.C., Wagstaff, S.J., Glenny, D. & Lockhart, P.J. (2002) Plant dispersal NEWS from New Zealand. *Trends in Ecology and Evolution*, **17**, 514-520.

## Supplementary Materials

**Appendix 1.** Data set used in the phenetic analysis of *Sicyos* in Australia, New Zealand, and the south-western Pacific. See Table 1 for characters relating to numbers in the header row. See Table 2 for voucher data.

	Characters														
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NZ1	24	9	4.5	10.2	5.3	5.8	42	5.8	93	66	14	16.0	12.8	1.5	2.3
NZ2	23	8	4.2	10.0	5.4	5.4	50	6.0	135	95	25	10.9	8.7	1.5	2.4
NZ3	25	8		9.8	6.2	4.5	46	6.2	124	72	17	14.5	10.6	1.3	2.4
NZ4	26	8	4.3	8.5	6.0	4.2	53	6.4	152	85	35	11.0	10.4	1.8	2.2
NZ5	25	8	4.8	9.2	6.2	5.3	48	5.5	110	72	18	12.4	9.2	1.5	2.0
NZ6	14	10		7.8	3.8	4.8	26	4.8	37	22	8	4.8			
NZ7	12	7	3.0	7.2	3.2	4.2	27	4.2	53	42	6	5.0			
NZ8	25	9		10.5	6.5	4.6	52	6.5	140	88	19	12.0	10.0	2.2	2.8
NZ9	12	10	4.3	8.0	3.8	4.2	20	4.0	55	22	11	4.2	8.2	1.4	1.7
NZ10	16	8		8.8	3.6	3.8	17	4.8	106	68	21	4.5			
NZ11	24	14	4.0	11.3	6.5	4.5	38	6.5	116	65	22	11.2	11.8		
NZ12	34	15	4.8	11.8	6.7	5.5	27		104	78	15	12.5	10.0		
KI1	24	8	4.5	11.8	6.0	5.5	36	6.8	110	79	16	12.3	10.5		
KI2	18	8		9.0	5.8	4.6	40	6.0	92	84	23	9.5	10.6	1.3	2.4
KI3	23	9		9.2	5.2	4.8	56	6.5	126	52	22	10.8	10.2	2.0	1.9
NI1	23	9	2.6	7.8	3.7	7.0	25	4.8	124	93	13	9.3	4.5		
NI2	16	8	2.5	7.2	3.2	5.5	24		61	49	9	6.5	5.0		
LH1	22	10	3.0	7.8	3.5	6.5	24	4.7	62	47	8	6.2	6.8		
LH2	28	8		7.2	3.3	5.5	28		78	61	10	6.0	6.2		
LH3	17	7		7.0	3.0	6.9	26	4.4	80	36	14	5.8	5.8		
TS1	10	8	3.4	7.8	3.7	6.0	23		57	35	11	5.2	6.3	1.8	2.2
TS2	11	10	2.8	7.3	3.5	7.5	22	4.6	68	40	12	3.5	6.0		
VG1	21	7	2.7	7.2	3.5	6.0	23	4.5	79	67	12	5.0	6.5		
VG2	14	8	2.5	6.5	3.0	5.8	21		67	50	15	3.5	5.8	1.5	1.8
NS1	13	6		5.5	2.7	4.5	19	4.0	60	53	7	4.8	7.4	1.8	1.7
NS2	23	7	2.8	7.5	3.6	7.0	28	4.8	104	93	12	8.5	6.4	1.5	1.7
NS3	15	8	2.4	8.2	3.8	3.5	22	5.0	87	65	11	6.8	6.0		
QL1	13	10	2.4	7.0	3.7	3.5	28	4.2	90	78	13	6.5	5.3		
QL2	15	6	3.0	7.5	3.8	6.5	21		79	55	9	4.5	5.8		
QL3	17	7		6.5	3.5	6.8	24	4.0	114	89	11	6.8	5.3		
QL4	11	7	2.5	8.3	4.0	7.0	22		93	75	12	4.0	4.2	1.6	1.7
QL5	36	5	2.0	4.8	2.8	2.6	13	3.5	60	37	14	9.6	4.2	1.1	0.8
QL6	27	4		5.3	3.0	2.8	11		34	27	12	6.0	3.7	0.9	0.7
QL7	57	6	2.4	4.7	2.9	2.8	12	3.2	95		22	11.3	4.8	0.9	0.7

## General Discussion

### Morphology, monophyly and sampling

In both of my study groups, molecular data has shown that traditional, morphology-based concepts did not result in monophyletic groups, and that numerous smaller genera need to be incorporated in *Cucumis* and *Sicyos* if monophyly was to be established. Reasons for these ‘unnatural’ genus concepts may be attributed to the choice of characters that circumscribe the groups. As vegetative parts, especially leaves, are morphologically highly variable even within species of Cucurbitaceae, and floral characters are often not easily preserved because the flowers are thick-fleshy and do not dry well, classifications for both of my focal clades often relied on fruit morphological characters. In *Cucumis*, even in its old circumscription, attempts to construct a key using only floral and inflorescence characters have failed (Kirkbride, 1993). In the case of *Sicyos*, my results show that certain fruit traits like wings or spines have arisen or been lost several times across the phylogeny, making the traditional genera poly- or paraphyletic, and in *Cucumis*, the red fruits and typically shaped seeds once thought to delimitate species of *Mukia* and *Dicaelospermum* form a clade that is nested within *Cucumis*.

Finding acceptance for molecular phylogenetic results that turn over long-established taxonomic concepts is sometimes difficult. Thus, despite convincing and well-supported results from molecular phylogenetic analyses, some taxonomists prefer to keep using the traditional classification, which they feel is easier (de Wilde & Duyfjes, 2010), probably because they have memorized it in the course of their careers. The exceptional difficulties in deducing Cucurbitaceae relationships from morphology are reflected by the fact that, prior to revisions in a broad molecular phylogenetic framework, 50 out of the 130 genera of Cucurbitaceae contained a single species (Kocyan *et al.*, 2007). Nevertheless, the issue of conflicting morphological and molecular classifications is by no means restricted to the Cucurbitaceae. With constant reshaping of our understanding of angiosperm evolution through molecular systematics, circumscriptions of many families or genera have changed drastically within the last years (Angiosperm Phylogeny Group, 1998, 2003). A prime example are the Scrophulariaceae, where genera had been moved back and forth between families based on morphological characters, and now DNA sequencing has radically changed the family’s circumscription again (Olmstead *et al.*, 2001; Albach *et al.*, 2005; Oxelman *et al.*, 2005), leaving a lot for older botanists to relearn. Incoming students, of course, will simply learn the new classification.

Only comprehensive and geographically unbiased sampling, including as many of the presumed members of a clade as possible, can lead to a good understanding of the evolutionary processes that occurred during a clade's history. In *Sicyos*, my dense species sampling permitted identifying the geographic origin of the species on Hawaii, the Galápagos, Australia, and New Zealand, and including more than just the type species of all genera suspected to be part of or close to *Sicyos* showed that the majority of them were not only embedded in *Sicyos* but also paraphyletic themselves. In *Cucumis*, species from five other genera were found to be embedded in the genus (Renner *et al.*, 2007), and including these genera (to make it a monophyletic group) led to a radical revision of its biogeographic scenario, with now almost as many species in Asia and Australia as there were originally known from Africa (Sebastian *et al.*, 2010a). Dense sampling in my study on *Cucumis* led to the discovery of an unexpectedly large species diversity in Asia and Australia partly because supposedly widespread species thought to range from Africa across Asia to Australia turned out to comprise several genetically and morphologically distinct lineages. Such wide distributions would have required rapid recent range expansion (found for example in the many cucurbit species that are distributed by humans, such as *Momordica charantia* or *Lagenaria siceraria*) or a rather unlikely scenario of ancient dispersal with constant gene flow between very distant populations.

It is likely that more Cucurbitaceae species will come to light with continued analyses of more material that is still “hiding” in local herbaria in Asia, Australia and Africa that do not usually send loans, and are rarely visited by specialists from other parts of the World, such visits still being the most efficient way of finding new species (much more efficient than fieldwork). However, more fieldwork in areas with limited access also probably will continue to turn up new species. For example, the botanical exploration of Australia is far from complete, and collections, for the greatest part, have been only made along roadsides or in tourist areas. Fieldwork by one of my lab colleagues in India last year (N. Filipowicz, 2010) led to the discovery of yet another apparently new species of *Cucumis* with black fruits that is close to *C. ritchiei* and needs further investigation. A more detailed survey of the Indian herbaria and more fieldwork in that country (so far difficult due to many restrictions) would almost certainly reveal additional species. Similarly, the cucurbit flora of New Guinea, some of the Indonesian islands, Myanmar, and Bangladesh is still very poorly known, and more fieldwork is needed in those regions.

Most species of *Cucumis* and *Sicyos* have highly seasonal growth and lack aboveground parts during a large part of the year, which is why wild cucurbits from remote

areas generally tend to be undercollected (Gentry, 1991). Many species are still only known from one or very few collections. An example is *Cucumis debilis*, which in 2007 was described as a new species from the only existing collection of 1931 from Vietnam (de Wilde & Duyfjes, 2007). This also highlights the importance of museum collections, which in the case of herbaria have recently been inferred to harbor thousands of undescribed species (Bebber *et al.*, 2010). Herbaria also have an almost untapped potential to achieve little-biased geographical sampling at a relatively low cost, at least as long as fresh material, such as seeds or seedlings for chromosome counts, is not needed. Molecular techniques for DNA isolation from small fragments of very old collections (e.g., 172 years, Schaefer *et al.*, 2009; Sebastian *et al.*, 2010a, b) are generally successful in Cucurbitaceae (unfortunately other plant groups like orchids, Melastomataceae or Lauraceae are not so easy to work with). As illustrated by our sequencing of the sole known specimen of *Sicyos villosus* collected by Charles Darwin himself, single collections of species made almost 200 years ago can still shed light on the indigenous composition of biota like the Galápagos archipelago before their decline or alteration through intense human settlement, and along with this the destruction of habitats or introduction of pathogens. As centers of cucurbit diversity lie in the tropics and subtropics, including areas like Southeast Asia and Madagascar, where habitat destruction is progressing at an extremely rapid pace, and access to many areas for collections of plant material is increasingly impeded by government regulations, use of herbarium specimens may gain even more importance for future researchers.

### **Geographical distribution and dispersal**

As already outlined in the introduction, Cucurbitaceae have undergone many long-distance dispersals during their evolution, and this is also apparent in the geographical distribution of both of my study clades. In their revised monophyletic circumscriptions, they occur in all the major centers of Cucurbitaceae diversity, *Sicyos* is most abundant in the Neotropics, where 40% of cucurbits occur (Schaefer *et al.*, 2009), while *c.* 30 species of *Cucumis* occur in Africa and at least 25 in India, Indochina, the Malesian region, and Australia. Interestingly, neither clade dispersed across the Atlantic Ocean between Africa and South America. This cannot be attributed to climatic or ecological constraints, as species of *Cucumis* or *Sicyos* that were introduced to the respective regions by human transport established naturalized populations there. *Sicyos angulatus* has even become an invasive weed in many parts of the Old World (Hulina, 1996; Kurokawa *et al.*, 2009). Trans-Atlantic dispersal between Africa and South America or *vice versa* has occurred several times within the Cucurbitaceae – *Cayaponia*,

*Siolmatra*, and *Luffa* have this range disjunction (Schaefer *et al.*, 2009; Duchen & Renner, 2010), and such disjunctions are also known from *c.* 110 other genus-level groups of plants (Renner, 2004).

Only 2% of the 940-980 species of Cucurbitaceae (Schaefer & Renner, 2011) are native in Australia, and cucurbit species there occur in habitats from tropical rainforest to subtropical and temperate deciduous forest or savannas to the arid areas in the center of the continent. Both of my study groups have endemic species in Australia, and no fewer than nine of them were (re-)discovered through my work (Telford *et al.*, 2011, in review). Although new species of Cucurbitaceae continue to be discovered (previous section), species numbers of Australian lineages in general tend to be low. The largest Australian cucurbit radiation consists of the four species of *Austrobryonia* (Schaefer *et al.*, 2008). Most native Australian Cucurbitaceae have close relatives or even conspecifics among tropical Asian lineages, which is the case in *Cucumis*, *Diplocyclos*, *Muellerargia*, *Neoalsomitra*, *Zehneria* and *Trichosanthes* (Telford, 1982; De Wilde & Duyfjes, 2003, 2006; Schaefer *et al.* 2008). The seven Australian species of *Cucumis* arose from four dispersal events into that continent, all of them from Southeast Asia (Sebastian *et al.*, 2010a) and the oldest dating to the early Pliocene, *c.* 5 million years ago, a time when severe aridity set in due to an ever steeper latitudinal temperature gradient with the expansion of the Antarctic ice cap (Bowler, 1982; Frakes, 1999). None of the lineages that arrived in Australia dispersed back to Southeast Asia, and this is also the case in the other cucurbit lineages with source populations in Southeast Asia. Increasing aridification in the late Neogene and increasingly unstable, relatively small areas of tropical habitats probably established source-sink dynamics in favour of dispersal from the relatively stable areas of Southeast Asian rain forests into Australia (Kershaw *et al.*, 2005; Sniderman & Jordan, 2011), rather than in the other direction. An exception among native Australian Cucurbitaceae is *Sicyos*, which entered the Australian continent from the eastern edge of the Pacific Ocean by long-distance dispersal from the Neotropics (Sebastian *et al.*, in review). In addition to this, *Sicyos* is one of the few cucurbit lineages that spread further into the south-western Pacific, and it is the only cucurbit genus with native and endemic species in New Zealand.

Diaspore morphology plays an important role in dispersal ability of a plant species. The fruits of Cucurbitaceae have been evolutionarily highly plastic, and of the higher-ranked clades (i.e., tribes), none have a unique characteristic fruit type (Kocyan *et al.*, 2007). The majority of fruits are berry-like, pulpy with conspicuous colors of the exocarp, sometimes striped, banded or flecked, suggesting seed dispersal is by animals, especially birds, that feed

on them (Schaefer & Renner, 2011). Endozoochory is the most likely dispersal mode for *Cucumis*, and seeds of *Cucumis melo* subsp. *agrestis*, have been found in the stomachs of Australian bustards in the northern Australian savannas (see Schaefer *et al.*, 2008). I myself have watched birds feed on the fruits of *Cucumis hystrix* in Northern Thailand. In some other species, such as *Cucumis oreosyce*, fruits open explosively (also in *Muellerargia timorensis* from the sister genus, which is commonly called ‘spitting gourd’), in others, fruits mature below ground and are then dug up by animals, such as aardvarks (*Orycteropus afer*; Meeuse, 1958), and *Cucumis messorius* is dispersed by harvesting ants (see Renner *et al.*, 2007). In one newly discovered species from Australia, the developing fruit is pushed into rock crevices or under foliage on the ground by the elongating pedicel and thus often matures hidden from animals like birds that could feed on them (my observation in Kakadu National Park, NT, Australia). The evolution of small fruits, which usually turn red at maturity, as is typical of fruits adapted for bird dispersal, in the *C. maderaspatanus/C. ritchiei* clade appears to have coincided with increased dispersal ability across Southeast Asia, to Australia and even back to Africa. In *Sicyos*, on the other hand, long-distance dispersal was always associated with the presence of retrorsely barbed spines on the surface of small, dry one-seeded fruits, which appear adapted to exozoochory. Lineages of Sicyoeae that evolved larger, fleshy, and glabrous fruits more likely to be eaten by animals did not get dispersed across very long distances and are mostly restricted to Central America. Interestingly, winged fruits, which evolved in two *Sicyos* lineages, also did not result in LDD between continents. This is in contrast to other cucurbit lineages (e.g., *Zanonia/Siolmatra*, *Neoalsomitra*), and lineages in other plant families (e.g., Asteraceae), where wind-dispersed groups often appear to be efficient dispersers. However, it may be worthwhile to recall that morphologically defined dispersal syndromes and long-distance dispersal are not always correlated (Higgins *et al.*, 2003), and that selection can never act in favor of transoceanic dispersal. Long-distance dispersals are, by definition, non-standard events, that may sometimes be favored by certain morphological adaptations of the diaspore but one cannot predict which species will be long-distance dispersers and which will not.

### **Implications for cultivated species**

The past years have seen significant progress in cucurbit genetics and genomics research, the biggest achievement probably being the sequencing of the nuclear genomes of three *Cucumis sativus* cultivars (Huang *et al.*, 2009; Cavagnaro *et al.*, 2010; Woycicki *et al.*, 2011). These three cucumber genome sequences provide exciting opportunities to address a number of

fundamental issues as well as advance traditional cucumber breeding with applied genomics tools. Already, the cucumber total genome has helped to solve the long-standing question of the processes that led to the transition from twelve chromosome pairs in all *Cucumis* species that have had their chromosomes counted to seven pairs in *C. sativus*. This has been achieved through studies of the synteny among parts of the genomes of *C. melo*, *C. sativus*, and a more distant relative, *Citrullus lanatus*, the watermelon (Huang *et al.*, 2009; Li *et al.*, 2011). Six of the seven *C. sativus* chromosomes are the result of fusion of two chromosomes each in the melon genome; the seventh chromosome (*C. sativus* chromosome 1) appears to go back at least to the most recent common ancestor of *Citrullus* and *Cucumis* (Huang *et al.*, 2009). Soon, whole genome sequences of melon (Benjak *et al.*, 2010) and watermelon (Xu *et al.*, 2009) will be available, and an alignment of these total genomes should provide even more insights into the evolutionary history of cucurbit genomes. However, to more fully understand the chromosome fusions and inter-chromosome rearrangements that occurred after the split between *C. melo* and *C. sativus* it will be helpful to study the chromosomes of the 22 species that are more closely related to *C. sativus* than is *C. melo* (Sebastian *et al.*, 2010a, Fig. 2). I have recently counted the chromosomes of *C. debilis* and *C. ritchiei* and found  $2n = 2x = 24$  chromosomes (P. Sebastian, unpublished data), but there are still 18 species close to *C. sativus* that have never been counted.

While melon is considered genetically diverse, cucumber is a crop with a narrow genetic base within domesticated market types (Staub *et al.*, 2008), and it is susceptible to many diseases and insect damage (Whitaker & Davis, 1996) resulting in heavy yield losses. In cucurbit breeding, natural sources of resistance to destructive viruses and other pathogens, especially fungi, are mostly lacking but resistance by means of genetic engineering has been implemented for several cucurbit crops (Kishimoto *et al.*, 2004; Gal-On *et al.*, 2005; Wu *et al.*, 2009). *Cucumis sativus* var. *hardwickii*, the wild progenitor of the cucumber and the only taxon that is cross-compatible with this cultivar, possesses some resistance to several highly problematic pathogens such as root-knot nematodes (Walters *et al.*, 1993), *Cucumber mosaic virus* (Munshi *et al.*, 2008). and downy mildew (Sharma & Hore, 1996). Several other wild species of *Cucumis* from Africa like *C. sagittatus*, *C. ficifolius*, *C. metuliferus*, and *C. anguria*, the latter two are minor local crops, have also been found to carry some of them (Walters *et al.*, 2006; National Research Council, 2008; Lebeda, 1984), but introgression into the commercial germplasm has been difficult (Ren *et al.*, 2009). The last common ancestor of species like *C. sativus* and *C. metuliferus* lived about twelve million years ago (Sebastian *et al.*, 2010a), yet this time interval already was sufficient for enough changes to occur in their

respective genomes to yield only 50% amplification of large datasets of cucumber simple sequence repeats (SSR's) in African *C. metuliferus*, while more than 80% could be amplified in the cucumber's sister species *C. hystrix* (Weng, 2010). At this time, we are still at the very beginning of bringing the newly found, less-distant relatives of cucumber and melon into cultivation, but detailed studies concerning their crossability or other potentials for crop improvement will need to be carried out before we can assess their direct value for traditional or modern techniques of crop breeding.

## References

- Albach, D.C., Meudt, H.M. & Oxelman, B. (2005) Piecing together the 'new' Plantaginaceae. *American Journal of Botany*, **92**, 297-315.
- Angiosperm Phylogeny Group (1998) An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden*, **85**, 531-553.
- Angiosperm Phylogeny Group (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society*, **141**, 399-436.
- Bebber, D.P., Carine, M.A., Wood, J.R.I., Wortley, A.H., Harris, D.J., Prance, G.T., Davidse, G., Paige, J., Pennington, T.D., Robson, N.K.B. & Scotland, R.W. (2010) Herbaria are a major frontier for species discovery. *Proceedings of the National Academy of Sciences USA*, **107**, 22169-22171.
- Benjak, A., Gonzalez, V., Mir, G., Arús, P., Aranda, M., Álvarez-Tejado, M., Droege, M., Du, L., Puigdomènech, P. & Garcia-Mas, J. (2010) A draft sequence of the melon genome revealed by high throughput shotgun sequencing. *Plant & Animal Genomes XVIII Conference abstracts*. Available at [http://www.intl-pag.org/18/abstracts/P01\\_PAG\\_XVIII\\_053.html](http://www.intl-pag.org/18/abstracts/P01_PAG_XVIII_053.html) (last accessed July 2011).
- Bowler, J.M. (1982) Aridity in the late Tertiary and Quaternary of Australia. *Evolution of the flora and fauna of arid Australia* (ed. by W.R. Barker and P.J.M. Greenslade), pp. 35-45. Peacock Publications, Glen Osmond, SA.
- Cavagnaro, P.F., Senalik, D.A., Yang, L., Simon, P.W., Harkins, T.T., Kodira, C.D., Huang, S. & Weng, Y. (2010) Genome-wide characterization of simple sequence repeats in cucumber (*Cucumis sativus* L.). *BMC Genomics*, **11**, 569.
- De Wilde, W.J.J.O. & Duyfjes, B.E.E. (2003) Revision of *Neosalsomitra*. *Blumea*, **48**, 99-121.
- De Wilde, W.J.J.O. & Duyfjes, B.E.E. (2006) *Mukia* Arn. (Cucurbitaceae) in Asia, in particular Thailand. *Thai Forest Bulletin (Botany)*, **34**, 38-52.
- De Wilde, W.J.J.O. & Duyfjes, B.E.E. (2007) The wild species of *Cucumis* L. (Cucurbitaceae) in South-East Asia. *Adansonia, sér. 3*, **29**, 239-248.
- De Wilde, W.J.J.O. & Duyfjes, B.E.E. (2010) Cucurbitaceae. *Flora Malesiana, ser. I – Seed Plants*, vol. 19 (ed. by H.P. Nooteboom). National Herbarium Nederland, Leiden.
- Dillehay, T.D., Rossen, J., Andres, T.C. & Williams, D.E. (2007) Pre-ceramic adoption of peanut, squash, and cotton in Northern Peru. *Science*, **316**, 1890-1893.
- Duchen, P. & Renner, S.S. (2010) The evolution of *Cayaponia* (Cucurbitaceae): repeated shifts from bat to bee pollination and long-distance dispersal to Africa 2-5 million years ago. *American Journal of Botany*, **97**, 1129-1141.
- Frakes, L.A. (1999) Evolution of Australian environments. *Flora of Australia*, vol. 1. *Introduction* (ed. by A.E. Orchard), pp. 163-203. CSIRO Publishing, Melbourne.
- Gal-On, A., Wolf, D., Antignus, Y. et al., 2005. Transgenic cucumbers harboring the 54-kDa putative gene for *Cucumber fruit mottle mosaic tobamovirus* are highly resistant to viral

- infection and protect non-transgenic scions from soil infection. *Transgenic Research*, **14**, 81-93.
- Gentry, A.H. (1991) The distribution and evolution of climbing plants. *The biology of vines* (ed. by F.E. Putz and H.A. Mooney), pp. 3-42. Cambridge University Press, Cambridge.
- Higgins, S.I., Nathan, R. & Cain, M.L. (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology*, **84**, 1945-1956.
- Huang, S., Ruiquiang, L., Zhang, Z., *et al.* (2009) The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics*, **41**, 1275-1281.
- Hulina, N. (1996) New dangerous weed in Croatia: *Sicyos angulatus* (Cucurbitaceae). *Poljoprivredna Znanstvena Smorta*, **61**, 259-264.
- Kershaw, A.P., Moss, P.T. & Wild, R. (2005) *Patterns and causes of vegetation change in the Australian Wet Tropics region over the last 10 million years*. Tropical rainforests: past, present, and future (ed. by E. Bermingham, C.W. Dick and C. Moritz), pp. 374-400. University of Chicago Press, Chicago.
- Kirkbride, J.H., Jr. (1994) Revision of *Cucumella* (Cucurbitaceae, Cucurbitoideae, Melothriaceae, Cucumerinae). *Brittonia*, **46**, 161-186.
- Kishimoto, K., Nishizawa, Y., Tabei, Y., Nakajima, M., Hibi, T. & Akutsu, K. (2004) Transgenic cucumber expressing an endogenous class III chitinase gene has reduced symptoms from *Botrytis cinerea*. *Journal of General and Plant Pathology*, **70**, 314-320.
- Kocyan, A., Zhang, L.-B., Schaefer, H. & Renner, S.S. (2007) A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution*, **44**, 553-577.
- Kurokawa, S., Kobayashi, H. & Senda, T. (2009) Genetic diversity of *Sicyos angulatus* in central and north-eastern Japan by inter-simple sequence repeat analysis. *Weed Research*, **49**, 265-372.
- Lebeda, A. (1984) Screening of wild *Cucumis* species for resistance to cucumber powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). *Scientia Horticulturae*, **24**, 241-249.
- Li, D., Cuevas, H.E., Yang, L., *et al.* (2011) Syntenic relationships between cucumber (*Cucumis sativus* L.) and melon (*C. melo* L.) chromosomes as revealed by comparative genetic mapping. *BMC Genomics*, **12**: 396. doi: 10.1186/1471-2164-12-396.
- Meeuse, A.J.D. (1958) A possible case of interdependence between a mammal and a higher plant. *Archives Néerlandaises de Zoologie*, **13**, 314-318.
- Munshi, A., Panda, B., Mandal, B. *et al.* (2008) Genetics of resistance to Cucumber mosaic virus in *Cucumis sativus* var. *hardwickii* R. Alef. *Euphytica*, **164**, 501-507.
- National Research Council (2008) Horned melon. *Lost crops of Africa*, vol. III: Fruits, pp. 89-101. The National Academic Press, Washington DC.
- Olmstead, R.G., dePamphilis, C., Wolfe, A.D., Young, N.D., Elisens, W.J. & Reeves, P.A. (2001) Disintegration of Scrophulariaceae. *American Journal of Botany*, **88**, 348-361.
- Oxelman, B., Kornhall, P., Olmstead, R.G. & Bremer, B. (2005) Further disintegration of Scrophulariaceae. *Taxon*, **54**, 411-425.
- Ren, Y., Zhang, Z., Liu, J. *et al.* (2009) An integrated genetic and cytogenetic map of the cucumber genome. *PLoS ONE*, **4**: e5795.
- Renner, S.S. (2004) Plant dispersal across the tropical Atlantic by wind and sea currents. *International Journal of Plant Sciences*, **165**, S23-S33.
- Renner, S.S., Schaefer, H. & Kocyan, A. (2007) Phylogenetics of *Cucumis* (Cucurbitaceae): cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evolutionary Biology*, **7**, 58.
- Sanjur, O.I., Piperno, D.R., Andres, T.C. & Wessel-Beaver, L. (2002) Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae)

- inferred from a mitochondrial gene: implications for crop plant evolution and areas of origin. *Proceedings of the National Academy of Sciences USA*, **99**, 535-540.
- Schaefer, H. & Renner, S.S. (2011) Cucurbitaceae. *Families and Genera of Vascular Plants* vol. 10. (ed. by K. Kubitzki), pp. 112–174. Springer Verlag, Berlin.
- Schaefer, H., Telford, I.R.H., & Renner, S.S. (2008) *Austrobryonia* (Cucurbitaceae), a new Australian endemic genus, is the closest living relative to the Eurasian and Mediterranean *Bryonia* and *Ecballium*. *Systematic Botany*, **33**, 125-132.
- Schaefer H., Heibl, C. & Renner, S.S. (2009) Gourds afloat: A dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 843–851.
- Sebastian, P., Schaefer, H., Telford, I.R.H. & Renner, S.S. (2010a) Cucumber (*Cucumis sativus*) and melon (*C. melo*) have their wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences USA*, **107**, 14269–14273.
- Sebastian, P., Schaefer, H. & Renner, S.S. (2010b) Darwin's Galapagos gourd: providing new insights 175 years after his visit. *Journal of Biogeography*, **37**, 975–980.
- Sebastian, P., Schaefer, H., Lira, R., Telford, I.R.H. & Renner, S.S. (in review) Radiation following long-distance dispersal: the contributions of time, opportunity, and diaspore morphology in *Sicyos* (Cucurbitaceae).
- Sharma, B.D. & Hore, D.K. (1996) Indian cucumber germplasm and challenges ahead. *Genetic Resources and Crop Evolution*, **43**, 7-12.
- Sniderman, J.M. & Jordan, G.J. (2011) Extent and timing of floristic exchange between Australian and Asian rain forests. *Journal of Biogeography*, **38**, 1445-1455.
- Staub, J.E., Robbins, M.D. & Wehner, T.C. (2008) Cucumber. Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae (ed. by J. Prohens and F. Nuez) pp. 241-282. Springer, New York.
- Telford, I.R.H. (1982) Cucurbitaceae. *Flora of Australia* vol. 8 (ed. by A.S. George), pp. 158–198, Australian Government Publishing Service, Canberra.
- Telford, I.R.H., Sebastian, P., Bruhl, J.J. & Renner, S.S. (2011) *Cucumis* (Cucurbitaceae) in Australia and eastern Malesia, including newly recognized species and the sister species of *C. melo*. *Systematic Botany*, **36**, 376-389.
- Telford, I.R.H., Sebastian, P., Bruhl, J.J. & Renner, S.S. (in review) *Sicyos* (Cucurbitaceae) in Australia, New Zealand and the islands of the south-western Pacific.
- Walters, S.A., Wehner, T.C. & Barker, K.R. (1993) Root-knot nematode resistance in cucumber and horned cucumber. *HortScience*, **28**, 151-154.
- Walters, S.A., Wehner, T.C., Daykin, M.E. & Baker, R.B. (2006) Penetration rates of root-knot nematode into *Cucumis sativus* and *C. metuliferus* roots and subsequent histological changes. *Nematropica*, **36**, 231-242.
- Weng, Y. (2010) Genetic diversity among *Cucumis metuliferus* populations revealed by cucumber microsatellites. *HortScience*, **45**, 214-219.
- Whitaker, T.W., & Davis, G.N. (1996) *Cucurbits – Botany, Cultivation, Utilization*. Interscience Publishers, New York.
- Woycicki, R., Gutman, J, Gawronski, P., *et al.* (2011) The genome sequence of the North-European cucumber (*Cucumis sativus* L.) unravels evolutionary adaptation mechanisms in plants. *PLOS ONE*, **6**: e22728.
- Wu, H.-W., Yu, T.-A., Raja, J.A.J., Wang, H.-C. & Yeh, S.-D. (2009) Generation of transgenic oriental melon resistant to *Zucchini yellow mosaic virus* by an improved cotyledon-cutting method. *Plant Cell Reports*, **28**, 1053-1064.
- Xu, Y., Guo, S.G., Zhang, H.Y., Gong, G.Y., Huang, S.W., Ye, H.P., Wu, M.Z., Zheng, Y. & Fei, Z.J. (2009) Latest advances in watermelon genomics. Abstract. 4<sup>th</sup> International Cucurbitaceae Symposium, (Sept. 20-24, Changsha, China), p.38.

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## Curriculum Vitae

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### Scientific work and education

- 06/2008 – current    **Ph.D. student, Systematic Botany and Mycology, University of Munich (LMU), Germany**  
Supervisor: Prof. Dr. Susanne S. Renner  
Project: “Phylogenetics and biogeography of two clades of Cucurbitaceae: one New World, one Old World, and both with economically important species”
- 12/2007 – 03/2008    **Research Assistant, Aquatic Ecology, University of Munich (LMU)**  
Project: Microscopic analysis of pelagic plankton samples
- 11/2007                **Diploma (M.Sc.) in Biology, University of Munich (LMU)**  
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- 10/2002 – 11/2007    **Studies of Biology at the University of Munich (LMU)**  
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### Grants and prizes

- 03/2009 – 02/2011    Ph.D. scholarship “Elite Network of Bavaria - Graduiertenförderung nach dem Bayerischen Eliteförderungsgesetz (BayEFG)”
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- 08/2010 Acceptance for participation in the DFG workshop for early career investigators “Systematics of Plants and Fungi”
- 06/2010 Travel grant for attendance of Cucurbitaceae 2010” conference, Charleston, SC, USA from “Elite Network of Bavaria”
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### Teaching

- 05/2011 – 07/2011 Student excursions for ‘Plant Identification’ course (LMU Munich)
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- 10/2008 – 11/2008 Teaching assistant ‘Botany and Mycology’ course ((LMU Munich)

### Field experience

- 03/2009 – 04/2009 Field collecting in Australia (Queensland and Northern Territory)
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### Talks

- Cucurbitaceae 2010:** ‘New relatives of cucumber and melon from Australia’. Charleston, South Carolina, November 14-18, 2010.
- 4<sup>th</sup> EES conference:** ‘Finding the wild relatives of melon, cucumber and chayote: molecular phylogenetics and biogeography of Cucurbitaceae’. LMU, Munich, October 12-13, 2010.
- Invited Seminar:** ‘The evolution of the cucumber (*Cucumis sativus*), the 6th angiosperm to have its genome sequenced’. Systematic Botany and Mycology, LMU Munich, May 27, 2009.

## List of Publications

### Papers

Telford, I.R.H., **Sebastian, P.**, Bruhl, J.J. and Renner, S.S. *Sicyos* (Cucurbitaceae) in Australia, New Zealand and the islands of the south-western Pacific. In review at *Australian Systematic Botany* (submitted August 26, 2011).

**Sebastian, P.**, Schaefer, H., Lira, R., Telford, I.R.H. and Renner, S.S. Radiation following long-distance dispersal: the contributions of time, opportunity and diaspore morphology in *Sicyos* (Cucurbitaceae). In review at *Journal of Biogeography* (submitted August 20, 2011).

Telford, I.R.H., **Sebastian, P.**, Bruhl, J.J. and Renner, S.S. *Cucumis* (Cucurbitaceae) in Australia and eastern Malesia including newly recognized species and the sister of *C. melo*. *Systematic Botany*, **36**, 376-389.

**Sebastian, P.**, Schaefer, H., Telford, I.R.H. and Renner, S.S. 2010. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences USA* 107(32): 14269-14273.

**Sebastian, P.**, Schaefer, H. and Renner, S.S. 2010. Darwin's Galapagos gourd: providing new insights 175 years after his visit. *Journal of Biogeography* 35(5): 975-978.

### Posters

**Sebastian, P.**, Schaefer, H., Telford, I.R.H. and Renner, S.S. Cucumber and melon have their wild progenitors in India, and the sister species of *Cucumis melo* is from Australia. VW Status Symposium in Evolutionary Biology. May 9-12, 2010, Fraueninsel, Germany. Abstracts p. 77.

Telford, I.R.H., **Sebastian, P.**, Schaefer, H., Bruhl, J.J. and Renner, S.S. *Cucumis* L. (Cucurbitaceae) in Australia: new relatives of cucumber and rock-melon. Australian Systematic Botany Society 2009 Conference, Dec 1-3, 2009, Armidale, Australia.

**Sebastian, P.**, Schaefer, H. and Renner, S.S. More new relatives of cucumber (*Cucumis sativus*) from Australia, Indochina, India, Mauritius, and the Seychelles. Systematics 2009, 7<sup>th</sup> Biennial Conference of the Systematics Association, Aug 10-14, 2009, Leiden, The Netherlands. Abstracts p. 120.

**Sebastian, P.**, Schaefer, H. and Renner, S.S. The origin of the Galapagos endemics *Sicyos* (*Sicyocaulis*) *pentagonus* and *Sicyos villosus* (Cucurbitaceae), the latter only collected by Darwin. Systematics 2009, 7<sup>th</sup> Biennial Conference of the Systematics Association, Aug 10-14, 2009, Leiden, The Netherlands, Abstracts p. 120.

**Sebastian, P.**, Lira, R., Cross, H.B., Telford, I.R.H., Motley T.J. and Renner, S.S. Biogeography of the *Sicyos* clade (Cucurbitaceae) – from the New World to Australia, New Zealand, and Hawaii. Botany 2009, July 25-29, 2009, Snowbird, Utah, USA.