Evolution and Loss of Long-Fringed Petals: A Case Study Using a Dated Phylogeny of the Snake Gourds, Trichosanthes (Cucurbitaceae)

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Evolution and loss of long-fringed petals: a case study using a dated phylogeny of the snake gourds, *Trichosanthes* (Cucurbitaceae)

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Abstract

**Background:** The Cucurbitaceae genus *Trichosanthes* comprises 90–100 species that occur from India to Japan and southeast to Australia and Fiji. Most species have large white or pale yellow petals with conspicuously fringed margins, the fringes sometimes several cm long. Pollination is usually by hawkmoths. Previous molecular data for a small number of species suggested that a monophyletic *Trichosanthes* might include the Asian genera *Gymnopetalum* (four species, lacking long petal fringes) and *Hodgsonia* (two species with petals fringed). Here we test these groups' relationships using a species sampling of c. 60% and 4759 nucleotides of nuclear and plastid DNA. To infer the time and direction of the geographic expansion of the *Trichosanthes* clade we employ molecular clock dating and statistical biogeographic reconstruction, and we also address the gain or loss of petal fringes.

**Results:** *Trichosanthes* is monophyletic as long as it includes *Gymnopetalum*, which itself is polyphyletic. The closest relative of *Trichosanthes* appears to be the sponge gourds, *Luffa*, while *Hodgsonia* is more distantly related. Of six morphology-based sections in *Trichosanthes* with more than one species, three are supported by the molecular results; two new sections appear warranted. Molecular dating and biogeographic analyses suggest an Oligocene origin of *Trichosanthes* in Eurasia or East Asia, followed by diversification and spread throughout the Malesian biogeographic region and into the Australian continent.

**Conclusions:** Long-fringed corollas evolved independently in *Hodgsonia* and *Trichosanthes*, followed by two losses in the latter coincident with shifts to other pollinators but not with long-distance dispersal events. Together with the Caribbean *Linnaeosicyos*, the Madagascan *Ampelosicyos* and the tropical African *Telfairia*, these cucurbit lineages represent an ideal system for more detailed studies of the evolution and function of petal fringes in plant-pollinator mutualisms.

Background

Deeply divided or fringed petal lobes are known from a range of angiosperm families, including Caryophyllaceae, Celastraceae, Cucurbitaceae, Myrtaceae, Orchidaceae, Saxifragaceae, and Tropaeolaceae [1]. While the origin and function of subdivided petals vary between groups, division of perianth edges is especially common among nocturnal hawkmoth-pollinated species (such as *Trichosanthes* [2], Figure 1), where the fringes, in combination with a light petal color, may enhance visibility and thus increase pollination success [3,4]. Experiments have shown that diurnal and nocturnal hawkmoths are attracted by floral scent but also rely on visual clues to find and recognize flowers even at extremely low light intensity [5,6]. A preference for high contrasts might help them find their nectar sources, and it seems plausible that fringed petals enhance the sharp contrast between the petal margin and a dark background [4].

In Cucurbitaceae, long-fringed petals are known in five genera that occur in Madagascar, tropical Africa, the Caribbean, and East and Southeast Asia [7,8]. The largest of them is *Trichosanthes* with currently 90–100 species of mainly perennial, 3 to 30 m long climbers that are usually dioecious and have medium-sized fleshy fruits. Referring to the petal fringes, Linnaeus formed the genus name from the Greek words for 'hair' (genitive...
Trichosanthes has its center of diversity in Southeast Asia, but ranges from India throughout tropical and subtropical Asia east to Japan, and southeast to New Guinea, Australia, and Fiji [9]. One species, the snake gourd, T. cucumerina L., is a widely cultivated vegetable in tropical and subtropical regions around the globe, and another 15 species are commonly used in Asian traditional medicine [10]. While floristic treatments are available for most of its range [9,11-16], a comprehensive revision of the nearly 300 names published in Trichosanthes is lacking (but see [17] for a synopsis).

Trichosanthes belongs in the tribe Sicyoeae, a group of 12 genera and c. 270 species that is supported by morphological and molecular data [18]. Based on a limited number of Trichosanthes species sequenced, it appeared that the genus might be paraphyletic, with the genera Gymnopetalum Arn. (four species; [19]) and Hodgsonia Hook.f. & Thomson (two species; [9]) possibly nested inside it [20]. Both share with Trichosanthes the white flowers, elongated receptacle-tubes, and free filaments. Hodgsonia also has long-fringed petals (Figure 2I), but differs from Trichosanthes and Gymnopetalum in its much larger fruits (up to 25 cm across) and unusual seeds. The petal margins in Gymnopetalum are entire (Figure 2A, 2E) or in one species shortly fimbriate [9]. Geographically, Gymnopetalum and Hodgsonia largely overlap with the distribution area of Trichosanthes except for their absence from New Guinea and Australia, and from much of the northeastern range of Trichosanthes (temperate China, Taiwan, Japan) [9].

Based on mainly fruit and seed characters, the 43 species of Trichosanthes occurring in the Flora Malesiana region have been grouped into six sections, the typical sect. Trichosanthes and sections Cucumeroides (Gaertn.) Kitam., Edulis Rugayah, Foliobracteola C.Y.Cheng & Yueh, Involucraria (Ser.) Wight, and Asterasperma W.J. de Wilde & Duyfjes [21,22]. The mainland Asian species, T. truncata C.B.Clarke, is in its own section, Truncata C.Y.Cheng & C.H.Yueh [23]. The four species of Gymnopetalum have been allocated to two sections that differ in flower morphology, the typical sect. Gymnopetalum with just one species from southern India and Sri Lanka and sect. Tripodanthera (M.Roem.) Cogn. with three southeast Asian and Malesian species [24].

Here we test the monophyly and phylogenetic placement of Trichosanthes using a broad sampling of some 60% of its species, including the type species of each section name, plus representatives of Gymnopetalum, Hodgsonia, and other Sicyoeae as well as more distant outgroups. The well-resolved phylogeny, combined with field observations on flower shape and color, allows us to test whether petal fringes in Old World Sicyoeae evolved just once as would be the case if Gymnopetalum and Hodgsonia were nested inside it [20] or multiple times as would be implied by these genera having separate evolutionary histories. A combination of molecular-dating and ancestral area reconstruction permits reconstructing the biogeographical history of the Trichosanthes clade.

**Results and discussion**

**Phylogenetic analyses and taxonomy**

Phylogenies obtained under Bayesian or Maximum Likelihood (ML) optimization revealed no statistically supported incongruences, defined as nodes with Bayesian posterior probabilities (PP) >0.95 or ML bootstrap support >75. A Bayesian consensus tree is shown in Figure 2. It reveals that the genus Trichosanthes is paraphyletic because Gymnopetalum is embedded in it, while Gymnopetalum is polyphyletic because its four species do not group together. Instead, G. tubiflorum (Wight & Arn.) Cogn. groups with species from sections Trichosanthes and Cucumeroides (1.00 PP/84 ML support), while G. orientale W.J.de Wilde & Duyfjes, G. chinensis (Lour.) Merr., and G. scabrum (Lour.) W.J. de Wilde & Duyfjes are sister to section Edulis (1.00 PP/86 ML). The Trichosanthes/Gymnopetalum clade (56 species sampled; 0.99 PP/62 ML support) is sister to Luffa, a genus of seven or eight species of which we included five. This sister group relationship, however, is only weakly supported (Figure 2). The genus Hodgsonia (two species with long-fringed flowers,
Figure 2 (See legend on next page.)
one sampled here) is only distantly related to the _Trichosanthes/Gymnopetalum_ clade.

Of the seven sections previously proposed in _Trichosanthes_ (see Background), three are supported by the molecular results, namely sections _Asterosperma_ (1.00 PP/100 ML; three species, two of them sampled here), _Cucumeroides_ (1.00 PP/93 ML; seven species, five sampled), and _Edulis_ (1.00 PP/75 ML; nine species, five sampled). Three other sections with more than one species (_Involucraria, Foliolbractoea, Trichosanthes_) are not monophyletic in their current circumscriptions. To achieve a more natural classification, a revised infrageneric classification has been proposed including two new sections [17].

The biogeographic history of the _Trichosanthes_ clade

Based on a fossil-calibrated Bayesian relaxed molecular clock model, _Trichosanthes_ originated during the Oligocene (Figure 3), an estimate influenced by our prior constraint of the crown node of the _Trichosanthes/Gymnopetalum_ clade to 34 Ma. This constraint is based on _Trichosanthes_-like seeds from the Upper Eocene of Bulgaria [25] dating to c. 34 Ma and seeds from the Oligocene of West Siberia [26] dating to c. 23.8 Ma [27]. Seeds assigned to _Trichosanthes_ have also been reported from Miocene and Pliocene sites in France, Germany, Italy, and Poland [28-30], and Pliocene _Trichosanthes_-like leaves are known from France [31]. The biogeographic analysis (Figure 4) inferred an East Asian origin of the genus (region C in Figure 4), but this inference is based only on the living species, while the just-discussed fossils indicate a more northern (Eurasian) range of _Trichosanthes_ before the global climate cooling at the end of the Oligocene. Many other extinct elements of the European Oligocene, Miocene, and Pliocene floras, such as _Taxodium_, _Craigia_, _Fagus kraeuselii_, _Ilex_, and tropical Araceae, such as _Caladiosoma_, also have nearest living relatives in tropical Southeast Asia [31,32].

Collision between the Eurasian and Australian tectonic plates started in the Late Oligocene, about 25 Ma ago, and the Sahul Shelf (carrying New Guinea) and Sunda Shelf (Sumatra, Java, and Borneo) reached their present proximity only by the Late Miocene, some 10 Ma [33,34]. Mid-Miocene pollen records indicate a warm, moist climate and rainforest expansion on these newly forming islands [35], allowing groups adapted to humid forest conditions, such as the liana clade _Trichosanthes_, to spread and diversify. Such plant groups would have benefited from land bridges that during times of sea level changes repeatedly connected New Guinea and Australia on the one hand, and Indochina, Sumatra, Java, and Borneo on the other. The lowest sea levels, during the last glacial maximum (LGM), were approximately 120 m below those of today, resulting in the complete exposure of the Sunda Shelf; even sea level reduction by just 40 m already connected Indochina, Sumatra, Java, and Borneo [35,36]. No land bridges, however, ever connected the islands on the Sunda Shelf with those in “Wallacea,” that is, Sulawesi, the Moluccas, and the Lesser Sunda Islands, or the latter with New Guinea and Australia on the Sahul Shelf. In zoogeography, these two boundaries are known as Wallace’s Line and Lydekker’s line, but their significance as floristic boundaries is doubtful [37,38].

The most striking transoceanic disjunctions in _Trichosanthes_ are numbered in Figure 4. They are (i) the disjunction between the Australian species _T. subvelutina_ F.Muell. ex Cogn. and its sister clade on the Asian mainland and areas of the Sunda Shelf, dated to 23.8 (29.4-18.4) Ma; (ii) the disjunction between _T. edulis_ Rugayag, _T. dentifera_ Rugayag, _T. laeoica_ C.Y.Cheng & L.Q.Huang, _T. schlechteri_ Harms from New Guinea, and _T. odontosperma_ W.E.Cooper & A.J.Ford from Australia on the one hand, and _Gymnopetalum chinense_, widespread in Asia as far East as Flores, and _G. orientale_ in Sulawesi, the Lesser Sunda Islands, and the Moluccas on the other (this is dated to 16.7 (22.1-11.2) Ma, but the position of _G. scabrum_ relative to _G. chinense_ and _G. orientale_ remains unclear; compare Figures 2, 3, and 4); and (iii) the disjunction between _T. wawrae_ Cogn. from Thailand, peninsular Malaysia, Sumatra, and Borneo, and its sister clade _T. papuana_ F.M.Bailey/T. pentaphylla F. Muell. ex Benth, from New Guinea and Australia, which dates to 7.1 (11.2-3.3) Ma.

_Trichosanthes_ range expansion between New Guinea and Australia occurred during the Pliocene/Pleistocene, when these two regions were repeatedly connected due to the above-mentioned sea level changes [36]. Thus, the estimated divergence time of the Australian species _T. odontosperma_ (a member of clade ii in Figure 4) from its New Guinean sister species, _T. edulis_, is 3.9 (6.4-1.6) Ma, while that of the sister species pair _T. papuana_ from...
the Aru Islands and New Guinea, and *T. pentaphylla* from Australia (clade iii in Figure 4) is 4.0 (7.1-1.4) Ma; considering their error ranges, these ages fall in the Pliocene/Pleistocene.

The geographic history of *T. pilosa* Lour. (including the synonyms *T. baviensis* Gagnep. and *T. holtzei* F.Muell. [16]), a widespread species here represented by seven samples from Queensland (Australia), Thailand, Vietnam, and Japan, cannot be inferred because the within-species relationships lack statistical support (Figure 2). Inferring the origin of the snake gourd, *T. cucumerina*, a vegetable cultivated in tropical and subtropical regions around the globe (represented by a single sample from Sri Lanka) also would require population-level sampling. Both species have fleshy red fruits and small seeds, probably dispersed by birds.

**Evolution and loss of petal fringes**

The phylogeny obtained here implies that long-fringed corollas evolved independently in the Asian genera *Hodgsonia* and *Trichosanthes* and were lost in three of the four species formerly placed in the genus *Gymnopetalum* (petals still bear c. 5 mm-long fringes in *G. orientale*). The two inferred losses (marked with an asterisk...
Figure 4 (See legend on next page.)
in Figure 2) coincide with shifts from nocturnal to diurnal flowering times (HS personal observation of *G. scabrum* and *G. chinense* in Cambodia, Jan. 2010, and China, Sept. 2005; N. Filipowicz, Medical University of Gdansk, personal observation of *G. tubiflorum* in India, Nov. 2010), and it therefore seems likely that there is a shift from predominantly nocturnal sphingid pollinators to diurnal bee or butterfly pollinators. The loss of fringes does not coincide with long-distance dispersal events to insular habitats (where hawkmoths might be absent), and the trigger for the pollinator shifts so far is unknown.

The adaptive function of the corolla fringes in pollinator attraction requires experimental study. An innate preference for radial patterns [39] and high contrasts might help hawkmoths find their nectar sources [5,6], and one possible explanation for the evolution of fringed petals is that they help create such a radial pattern and sharper contrasts between the petals and a dark background [4]. In a diurnal, hawkmoth-pollinated *Viola* species, more complex corolla outlines correlate with higher fruit set [40] but it remains to be tested if this is also the case in the nocturnal *Trichosanthes*-hawkmoth system. Another untested possibility is that the fringes with their highly increased surface area and exposed position might be involved in scent production [3,5,6] or produce a waving motion, in which waves are set up in the plant by pollinators (pers. comm., Feb. 2012) or produce a waving motion, and letters adjacent to taxon names correspond to the geographic origin of the sampled plant. Wallace’s Line is shown as a broken line between Borneo and Sulawesi, Lydekker’s Line is shown as a broken line between New Guinea and the Moluccas. The three numbered clades and inferred transoceanic disjunctions are discussed in the text.

Methods

**Morphology**

Herbarium specimens from A, BRI, CNS, E, GH, K, KUN, KYO, L, LE, M, MO, P, S, UC, UPS and US were obtained on loan or studied during herbarium visits. Determination of herbarium material was verified using identification keys [9,11-16,19,42]. All species in *Trichosanthes* have corolla fringes, and these are absent in three of the four *Gymnopetalum* species, except *G. orientale*, which can have short-fimbriate petal margins (fringes up to 5 mm length).

**Sampling, DNA extraction and amplification**

We included six DNA regions, namely the nuclear ribosomal ITS region (ITS1-5.8S-ITS2), the chloroplast genes *rbcL* and *matK*, the *trnL* and *trnL-trnF* intron and spacer, and *rpl20-rps12* spacer. Data for *rbcL* and the *trnL* region were taken from previous studies [7,18,20,43,44]. Only plant samples for which two or more markers were successfully sequenced were included in the analyses, and the combined dataset included one of the two species of *Hodgsonia*, all four of *Gymnopetalum*, and 52 of *Trichosanthes*, representing approximately 60% of the accepted species in the latter genus. Type species of all sections were included: *Gymnopetalum tubiflorum* (Wight & Arn.) Cogn. (G. sect. *Gymnopetalum*), *Gymnopetalum chinense* (Lour.) Merr. (G. sect. *Tripodantha*), *Trichosanthes postarii* W.J.de Wilde & Duyfjes (T. sect. *Asterosperma*), *Trichosanthes pilosa* Lour. (T. sect. *Cucumeroides*), *Trichosanthes edulis* Rugayah (T. sect. *Edulis*), *Trichosanthes kirilowii* Maxim. (T. sect. *Foliobracteola*), *Trichosanthes wallichiana* (Ser.) Wight (T. sect. *Involutaria*), *Trichosanthes villosa* Blume (T. sect. *Pseudovariifera*), *Trichosanthes cucumerina* L. (T. sect. *Trichosanthes*), *Trichosanthes truncata* C.B. Clarke (T. sect. *Truncata*), *Trichosanthes subvelutina* F. Muell. ex Cogn. (T. sect. *Villosae*). Species names and their authors, specimen voucher information, and GenBank accession numbers for all sequenced markers (including 262 new sequences) are summarized in Table 1. Total DNA was extracted using the Carlson/Yoon DNA isolation procedure [45] and a Mini-Beadbeater (BioSpec Products) to pulverize the plant material. Extracts were purified using the GE Illustra GFX™ PCR DNA and Gel Band Purification Kit following the standard protocol.

Conclusions

Molecular evidence supports the inclusion of *Gymnopetalum* into a then monophyletic *Trichosanthes* [17]. Our molecular phylogenies reveal that long-fringed petals evolved independently in *Hodgsonia* and *Trichosanthes/Gymnopetalum*, followed by two losses of corolla fringes in the latter clade, most likely associated with pollinator shifts. Molecular dating and a biogeographic analysis indicate an Oligocene initial diversification of *Trichosanthes* in mainland Asia. The lineage then diversified and spread in Malaysia (the Malesian biogeographic region) during the late Miocene and Pliocene, reaching the Australian continent several times.
### Table 1 Voucher information and GenBank accession numbers

| Species | No. | Voucher (Herbarium) | Origin of the sequenced material | ITS | rpl20-rps12 IS | matK | rbcL | trnL-trnF IS | trnL intron |
|---------|-----|---------------------|-----------------------------------|-----|---------------|------|------|---------------|-------------|
| Austrobryonia micrantha (F.Muell.) I.Telford | 1 | R. Telford 8173 (CANB) | Australia, New South Wales | EF487546 | EF487567 | EF487559 | EF487552 | EF487575 | EF487575 |
| Bryonia dioica Jacq. | 1 | S. Renner 2187 (M) | (1) Switzerland, cult. BG Zürich | (2) EU102709 | (1) DQ648157 | (1) DQ536641 | (1) DQ536791 | (1) DQ536791 | (1) DQ536791 |
| | 2 | A. Faure 661/76 (M) | Algeria, Lamoriciere | | | | | | |
| Cyclanthera pedata (L.) Schrad. | 1 | S. Renner et al. 2767 (M) | Germany, cult. BG Mainz | HE661293 | DQ648172 | DQ536667 | DQ535749 | DQ536767 | DQ536767 |
| Ecballium elaterium (L.) A.Rich. ssp. elaterium | 1 | M. Chase 922 (K) | (1) UK, cult. RBG-K | (2) EU102746 | (1) AY968541 | (1) AY973019 | (1) AY973023 | (1) AY973006 | (1) AY973006 |
| Echinocystis lobata (Michx.) Torr. & A.Gray | 1 | S. Renner et al. 2829 (M) | Germany, cult. BG Mainz | - | DQ648174 | DQ536673 | DQ535809 | DQ536814 | DQ536814 |
| Gymnopetalum chinense (Lour.) Merr. | 1 | H. Schaefer 2005/661 (M) | China, Guangdong | HE661294 | EU155612 | EU155606 | EU155601 | EU155621 | EU155630 |
| Gymnopetalum orientale W.J. de Wilde & Duyfjes | 3 | M. van Balgooy 7553 (L) | Indonesia, Bali | HE661301 | HE661468 | HE661397 | - | - | - |
| Gymnopetalum scabrum (Lour.) W.J. de Wilde & Duyfjes | 1 | W. de Wilde & B. Duyfjes 22269 (L) | Thailand, Central | HE661295 | DQ536556 | DQ536683 | DQ535754 | DQ536824 | DQ536824 |
| | 2 | J. Maxwell 16-11-2002 (CMU) | Thailand | HE661296 | HE661469 | HE661398 | - | - | - |
| Gymnopetalum scabrum (Lour.) W.J. de Wilde & Duyfjes | 3 | C.H. Wong, J. Helm & J. Schultze-Motel 2071 (LE) | China, Hainan | HE661297 | HE661470 | HE661399 | - | - | - |
| Gymnopetalum tubiflorum (Wight & Am.) Cogn. | 1 | N. Filipowicz & Z. Van Henwijnen NF25a (M) | India, Kerala | HE661298 | HE661471 | HE661400 | - | - | - |
| Gymnopetalum tubiflorum (Wight & Am.) Cogn. | 2 | A. Alston 1670 (UC) | Sri Lanka, Veragantota | HE661299 | HE661472 | HE661401 | - | - | - |
| Gymnopetalum tubiflorum (Wight & Am.) Cogn. | 3 | G.H.K. Thwaites CP1625 (K) | Sri Lanka | HE661300 | HE661473 | HE661402 | - | - | - |
| Hodgsonia heteroclitica Hook.f. & Thomson | 1 | P. Phonsena 4705 (L) | (1) Thailand, Nan | (1) HE661302 | (1) HE661474 | (1) HE661403 | - | (2) EU155631 - |
| | 2 | L. Loeffler s.n. (M) | Bangladesh | | | | | | |
| Lagenaria sicenaria (Molina) Standl. | 1 | M. Merello 1331 (MO) | Ghana | HE661303 | HE661475 | HE661404 | AY935747 | AY935788 | AY968570 |
| | 2 | S. Renner et al. 2767 (M) | seeds from D. S. Decker-Walters & A. Wagner TCN 1130 (FTG) | (1) Germany, cult. BG Munich, seeds from India, Ahmadnagar, Maharashtra | HE661305 | HE661476 | DQ536695 | DQ538262 | DQ536835 |
| Luffa acutangula (L.) Roxb. | 2 | S. Renner et al. 2767 (M) | China, cult. BG Guangzhou | HE661305 | HE661476 | DQ536695 | DQ538262 | DQ536835 | DQ536835 |
| | 3 | L.X. Zhou s.n. | China, cult. BG Guangzhou | | | | | | |

http://www.biomedcentral.com/1471-2148/12/108
| Plant Name                        | Collector          | Voucher Information                                                                 | GenBank Accession Numbers       |
|----------------------------------|--------------------|--------------------------------------------------------------------------------------|---------------------------------|
| **Luffa aegyptiaca Mill.**       | D.Z. Zhang         | 15 April 2003, China, cult. BG Guangzhou                                             | HE661306, HE661477, HE661405    |
| (incl. *L. cylindrica* L.)        |                    | no voucher                                                                            | DQ535827, DQ536836, DQ536836    |
| **Luffa echinata Roxb.**          | G. Schweinfurth    | 555 (M) Egypt                                                                         | HE661307, HE661478, HE661406    |
|                                  |                    | no voucher                                                                            | - EU436357, EU436357            |
| **Luffa graveolens Roxb.**        | S. Renner & A. Kocyan | 2758 (M), seeds from D. Becker-Walters 1543 (FTG 121855)                           | HE661308, EU436334, EU436409    |
|                                  |                    | Germany, cult. BG Munich, seeds from India, USDA PI540921                            | EU436385, EU436358, EU436358    |
| **Luffa quinquefolia** (Hook. & Arn.) Seemann | R. Berhaut | 7308 (M) Senegal                                                                       | HE661308, (1) EU436335, (2) DQ536697 |
|                                  |                    | (2) Germany, cult. BG Munich, seeds originally from Louisiana, USA                   | - (1) EU436359                  |
| **Marah macrocarpa** (Greene) Greene | M. Olson          | s.n. (MO) USA, Sonoran Desert                                                        | (1) USA, Sonoran Desert         |
|                                  |                    | 1009 (RSA) USA, Sonoran Desert                                                       | (2) USA, Sonoran Desert         |
| **Momordica charantia** L.        | S. Renner et al.   | 2775 (M) Germany                                                                      | HE661309, DQ491013, DQ491019    |
|                                  |                    | Germany, cult. BG Munich                                                              | DQ535760, DQ501269, DQ501269    |
| **Nothosorus suberosa** (F.M.Bailey) I.Telford | I. Telford     | 12487 (NE) Australia                                                                   | HE661310, DQ536575, DQ536709    |
|                                  |                    | SE Queensland                                                                         | DQ535762, DQ536844, DQ536844    |
| **Sicyos angulatus** L.           | M. Chase           | 979 (K) North America                                                                  | HE661311, DQ648189, DQ36732     |
| **Trichosanthes adhaerens**       | S. Lim, J. J. Postar & G. Markus | SAN 143273 (L) Malaysia, Borneo, Sabah                                              | HE661312, HE661479, - - - - - - |
| W.J. de Wilde & Dayfies           |                    |                                                                                      |                                 |
| **Trichosanthes auriculata** Rugayah | A. Kalat, J. Abdallah, & J. Clayton | BRUN 17016 (L) Borneo, Brunei                                                         | HE661313, HE661480, HE661407    |
|                                  |                    |                                                                                      | - - - - - -                     |
| **Trichosanthes bavensis** Gagnep. | N.M. Cuong        | 1248 (P) Vietnam                                                                       | HE661314, HE661481, - - - - - - |
|                                  |                    |                                                                                      |                                 |
| **Trichosanthes beccariana** Cogn. ssp. beccariana | W. de Wilde et al. | SAN 142229 (L) Malaysia, Borneo, Sabah                                                | HE661315, HE661482, HE661408    |
|                                  |                    |                                                                                      | - - - - - -                     |
| **Trichosanthes borneensis** Cogn. | C. Argent et al. | 93127 (E) Indonesia, Borneo, Kalimantan Timur                                         | HE661316, HE661483, - - - - - - |
|                                  |                    |                                                                                      |                                 |
| **Trichosanthes bracteata** (Lam.) Voigt | T. Haegele | 20 (M) India, Kochin                                                                   | HE661317, HE661484, EU155608    |
|                                  |                    |                                                                                      | EU155602, EU155622, EU155632    |
| **Trichosanthes celebica** Cogn.  | W. de Wilde & B. Dayfies | 21903 (L) Indonesia, Sulawesi                                                          | HE661318, HE661485, HE661409    |
|                                  |                    |                                                                                      | - - - - - -                     |
| **Trichosanthes cucumerina** L.   | H. Schaefer        | 2007/327 (M) Germany, cult. BG Munich                                                 | HE661319, EU155614, EU155609    |
|                                  |                    |                                                                                      | EU155603, EU155623, EU155633    |
| **Trichosanthes cucumerina** L.   | N. Lundqvist       | 11380 (UPS) Sri Lanka                                                                  | HE661320, HE661486, HE661410    |
|                                  |                    |                                                                                      | - - - - - -                     |
| **Trichosanthes dentifera** Rugayah | J.H.L. Waterhouse | 445-B (L) Papua New Guinea, Bougainville Is.                                          | HE661321, HE661487, - - - - - - |
|                                  |                    |                                                                                      |                                 |
| **Trichosanthes dioica** Roxb.    | O. Polunin, W. Sykes & J. Williams | S925 (E) Nepal                                                                         | HE661322, HE661488, HE661411    |
|                                  |                    |                                                                                      | - - - - - -                     |
| **Trichosanthes edulis** Rugayah   | W. Avé            | 4076 (L) Indonesia, Irian Jaya                                                        | HE661323, HE661489, HE661412    |
|                                  |                    |                                                                                      | - - - - - -                     |
| Scientific Name                  | Collection Details                  | Location Details                           | GenBank Accession Numbers | Notes |
|--------------------------------|-------------------------------------|--------------------------------------------|---------------------------|-------|
| *Trichosanthes elmeri* Merr.   | E.F.J. Campbell 43 (E)              | Malaysia, Borneo, Sabah                   | HE661324, HE661490        |       |
| *Trichosanthes globosa* Blume  | W. de Wilde et al. SAN 144003 (L)   | Malaysia, Borneo, Sabah                   | HE661325, HE661491, HE661413 |       |
| *Trichosanthes holtzei* F.Muell.| B. Gray 7482 (CNS)                 | Australia, N Queensland                   | HE661326, HE661492, HE661414 |       |
| *Trichosanthes homophylla* Hayata | Y.-C. Kao 499 (GH)                | Taiwan                                     | HE661327, HE661493, HE661415 |       |
| *Trichosanthes hylonoma* Hand.-Mazz. | Wuling Mt Exp 1646 (KUN)       | China                                      | HE661328, HE661494, HE661416 |       |
| *Trichosanthes intermedia* W.J. de Wilde & Duyfjes | V. Julaih et al. S 76602 (L)     | Malaysia, Borneo, Sarawak                 | HE661329, HE661495        |       |
| *Trichosanthes inthanonensis* Duyfjes & Pruesapan | 1. P. Phonsena, W. de Wilde & B. Duyfjes 3993 (L) | Thailand, Chiang Mai                   | HE661330, HE661496, HE661417 |       |
| *Trichosanthes inthanonensis* Duyfjes & Pruesapan | 2. K. Pruesapan et al. 67 (L)     | Thailand, Kanchanaburi                  | HE661331, HE661497, HE661418 |       |
| *Trichosanthes kirrii* Craib      | P. Phonsena, W. de Wilde & B. Duyfjes 3999 (L) | Thailand, Nan                           | HE661333, HE661498        |       |
| *Trichosanthes kinabaluensis* Rugayah | J. Postar et al. SAN 144260 (L)   | Malaysia, Borneo, Sabah                   | HE661334, EU155615, HE661419 | EU155624, EU155634 |
| *Trichosanthes kiniiwii* Maxim. var. japonica (Miq.) Kitam. | 3. H. Takahashi 20711 (GIFU)   | Japan                                      | HE661335, DQ536603, DQ536742 | DQ535855, DQ536874, DQ536874 |
| *Trichosanthes kiniiwii* Maxim. var. japonica (Miq.) Kitam. | 1. K. Kondo 05090401e (KYO)     | Japan                                      | HE661332, HE661499, HE661420 |       |
| *Trichosanthes kiniiwii* Maxim. var. japonica (Miq.) Kitam. | 2. K. Deguchi, K. Uchida, K. Shin & H. Hideshima s.n. (KYO) | Japan | - | HE661500, HE661421 |       |
| *Trichosanthes laceribractea* Hayata | 1. S. Fujii 9623 (KYO)           | Japan                                      | HE661336, HE661501, HE661422 |       |
| *Trichosanthes laceribractea* Hayata | 2. S. Fujii 9978 (KYO)           | Japan                                      | HE661337, HE661502, HE661423 |       |
| *Trichosanthes laceribractea* Hayata | 3. Liang Deng 7090 (KUN)         | China                                      | HE661338, HE661503        |       |
| *Trichosanthes laeoica* C.Y.Cheng & L.Q.Huang | 1. M. Coode et al. NGF 32585 (E)  | Papua New Guinea, Eastern Highlands       | HE661339, HE661504        |       |
| *Trichosanthes laeoica* C.Y.Cheng & L.Q.Huang | 2. P. Kati LAE 77807a (BRI)     | Papua New Guinea                          | HE661340, HE661505        |       |
| *Trichosanthes lepiniana* (Naud.) Cogn. | 1. J.D.A. Stainton 8522 (E)     | Nepal                                      | HE661341, HE661506, HE661424 |       |
| *Trichosanthes lepiniana* (Naud.) Cogn. | 2. Shanzu Wen 85 (KUN)          | China                                      | HE661342, HE661507, HE661425 |       |
| *Trichosanthes lepiniana* (Naud.) Cogn. | 3. H. de Boer HB49, coll. 1865 (P) | France, cult BG Paris                   | HE661343, HE661508        |       |
| Species                        | Voucher Information                                      | Country/Region                          | GenBank Accessions       |
|-------------------------------|----------------------------------------------------------|-----------------------------------------|--------------------------|
| Trichosanthes miyagii Hayata  | T. Yamazaki 310 (KYO)                                     | Japan                                   | HE661344 HE661509 HE661426 - - - |
| Trichosanthes montana Rugayah ssp. crassipes W.J. de Wilde & Duyfjes | J. Postar et al. SAN 144259 (L)                           | Malaysia, Borneo, Sabah                | HE661346 EU155616 HE661427 - EU155625 EU155635 |
| Trichosanthes montana Rugayah ssp. montana | W. de Wilde et al. 22279 (L)                           | Indonesia, Java                        | HE661345 HE661510 - - - |
| Trichosanthes mucronata Rugayah   | W. de Wilde & B. Duyfjes SAN 139459 (L)                | Malaysia, Borneo, Sabah                | HE661347 HE661511 HE661428 - - - |
| Trichosanthes multiflora Miq. 1 | S. Tsugaru, G. Murata & T. Sawada s.n. (KYO)            | Japan                                   | HE661348 HE661512 HE661429 - - - |
| Trichosanthes multiflora Miq. 2 | S. Fujii 9957 (KYO)                                      | Japan                                   | HE661349 HE661513 HE661430 - - - |
| Trichosanthes nervifolia L.    | B. Jonsell 3828 (UPS)                                    | Sri Lanka                               | HE661350 HE661514 HE661431 - - - |
| Trichosanthes obscura Rugayah   | K.M. Wang 1581 (L)                                       | Borneo, Brunei                         | HE661351 HE661515 - - - |
| Trichosanthes odontosperma W.E.Cooper & A.J.Ford | H. Schaefer 2007/09 (M)                             | Australia, Queensland                   | HE661352 EU037013 HE661432 - EU037011 EU037010 |
| Trichosanthes odontosperma W.E.Cooper & A.J.Ford | B. Gray 9147 (UPS)                                    | Australia, Queensland                   | HE661353 HE661516 HE661433 - - - |
| Trichosanthes odontosperma W.E.Cooper & A.J.Ford | I. Telford 11285 (CNS)                                   | Australia, Queensland                   | HE661354 HE661517 HE661434 - - - |
| Trichosanthes pallida Duyfjes & Pruesapan | P. Phonsena, W. de Wilde & B. Duyfjes 4658 (L)         | Thailand, Phetchaburi                  | HE661355 HE661518 HE661435 - - - |
| Trichosanthes pallida Duyfjes & Pruesapan | P. Phonsena, W. de Wilde & B. Duyfjes 3981 (L)         | Thailand, Phetchaburi                  | HE661356 HE661519 HE661436 - - - |
| Trichosanthes papuana F.M.Bailey | W. Takeuchi & O. Arna 17069 (L)                         | Papua New Guinea                       | HE661357 HE661520 HE661437 - - - |
| Trichosanthes pedata Merr. & Chun | Jiangiang Li 239 (KUN)                                 | China                                   | HE661358 HE661521 HE661438 - - - |
| Trichosanthes pendula Rugayah   | J. Postar et al. 144100 (L)                             | Malaysia, Borneo, Sabah                | HE661359 EU155617 HE661439 - EU155626 EU155636 |
| Trichosanthes pentaphylla F.Muell. ex Benth. | W. Cooper 2094 (CNS)                                   | Australia, Queensland                   | HE661360 HE661522 HE661440 - - - |
| Trichosanthes pentaphylla F.Muell. ex Benth. | W. Cooper 2061 (CNS)                                   | Australia, Queensland                   | HE661361 HE661523 HE661441 - - - |
| Trichosanthes phonsenae Duyfjes & Pruesapan | P. Phonsena, W. de Wilde & B. Duyfjes 4419 (L)      | Thailand, Phetchaburi                  | HE661362 HE661524 HE661442 - - - |
| Trichosanthes phonsenae Duyfjes & Pruesapan | P. Phonsena, W. de Wilde & B. Duyfjes 3980 (L)    | Thailand, Phetchaburi                  | HE661363 HE661525 HE661443 - - - |
| Species                                      | Voucher Information | GenBank Accession Numbers |
|----------------------------------------------|---------------------|----------------------------|
| *Trichosanthes pilosa* Lour.                | H. Schaefer 2007/17 (M) | HE661364 EU155620 EU155611 |
|                                              | Australia, Queensland | EU155629 EU155639          |
| *Trichosanthes pilosa* Lour.                | P. Phonsena, W. de Wilde & B. Duyfjes 3913 (L) | HE661365 HE661526 HE661444 |
|                                              | Thailand, Chiang Mai  | -                            |
| *Trichosanthes pilosa* Lour.                | H. Takahashi 20755 (GIFU) | DQ536604 DQ536743 DQ538586 |
|                                              | Japan                | DQ536875 DQ536875           |
| *Trichosanthes pilosa var. roseipulpa* W.J. de Wilde & Duyfjes | P. Phonsena, W. de Wilde & B. Duyfjes 4694 (L, holotype) | HE661367 HE661529 HE661446 |
|                                              | Thailand, Nan        | -                            |
| *Trichosanthes postani* W.J. de Wilde & Duyfjes | J. Postar et al. SAN 144066 (L, isotype) | HE661368 EU155618 HE661447 |
|                                              | Malaysia, Borneo, Sabah | EU155627 EU155637           |
| *Trichosanthes postani* W.J. de Wilde & Duyfjes | J. Postar et al. SAN 144098 (L) | HE661369 HE661530 HE661448 |
|                                              | Malaysia, Borneo, Sabah | -                            |
| *Trichosanthes pubera* Blume ssp. rubriflos (Cayla) Duyfjes & Pruesapan var. fissisepala Duyfjes & Pruesapan | P. Phonsena, W. de Wilde & B. Duyfjes 4451 (L) | HE661370 HE661531 HE661449 |
|                                              | Thailand, Chiang Mai  | -                            |
| *Trichosanthes pubera* Blume ssp. rubriflos (Cayla) Duyfjes & Pruesapan var. fissisepala Duyfjes & Pruesapan | K. Pruesapan et al. 56 (L) | HE661371 HE661532 HE661450 |
|                                              | Thailand, Kanchanaburi | -                            |
| *Trichosanthes pubera* Blume ssp. rubriflos (Cayla) Duyfjes & Pruesapan var. rubriflos | R. Zhang 1 (M) | HE661372 DQ536560 DQ536888 |
|                                              | China, cult. South China BG, Guangzhou | DQ53819 DQ536828 -          |
| *Trichosanthes pubera* Blume ssp. rubriflos (Cayla) Duyfjes & Pruesapan var. rubriflos | P. Phonsena, W. de Wilde & B. Duyfjes 3907 (L) | HE661373 HE661533 HE661451 |
|                                              | Thailand, Saraburi    | -                            |
| *Trichosanthes quinquangulata* A.Gray       | P. Phonsena, W. de Wilde & B. Duyfjes 4416 (L) | HE661374 HE661534 HE661452 |
|                                              | Thailand, Phetchaburi | -                            |
| *Trichosanthes quinquangulata* A.Gray       | N. Koonthudthod et al. 326 (L) | HE661375 HE661535 HE661453 |
|                                              | Thailand, Phetchaburi | -                            |
| *Trichosanthes quinquefolia* C.Y.Wu          | K. Nanthavong et al. BT 705 (L) | HE661376 HE661536 HE661454 |
|                                              | Laos, Khammouan       | -                            |
| *Trichosanthes reticulinervis* C.Y.Wu ex S.K.Chen | X.F. Deng 131 (IBSC) | HE661377 DQ536605 DQ536744 |
|                                              | China, Guangdong      | DQ538587 DQ536876 DQ536876 |
| *Trichosanthes rosthornii* Harms             | Jingliang Chuan 5654 (KUN) | HE661378 HE661537 HE661455 |
|                                              | China                 | -                            |
| *Trichosanthes rosthornii* Harms             | A. Henry 1626 (LE)    | HE661379 HE661538 -         |
|                                              | China, Hubei          | -                            |
| *Trichosanthes schlechteri* Harms           | W. Takeuchi & D. Ama 15663 (LAE) | HE661380 EU155619 EU155610 |
|                                              | Papua New Guinea      | EU155605 EU155628 EU155638  |
|                                              | J. Postar et al. SAN 151201 (L) | HE661381 HE661539 -         |
|                                              | Malaysia, Borneo, Sabah | -                            |
| Species and Voucher Information | GenBank Accession Numbers |
|---------------------------------|---------------------------|
| *Trichosanthes sepilokensis*    | Rugayah                   |
| C.Y.Wu                          | Qiuwu Wang 85620 (KUN)    | China | HE661382 | HE661540 | - | - | - | - |
|                                 |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes smilacifolia*    |                           |       |         |         |       |   |   |   |   |
| F.Muell. ex Cogn.               |                           |       |         |         |       |   |   |   |   |
| 1 I. Telford 9778 (CANB)        |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes subvelutina*     |                           |       |         |         |       |   |   |   |   |
| F.Muell. ex Cogn.               |                           |       |         |         |       |   |   |   |   |
| 2 F. Davies 1541 (CANB)         |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes subvelutina*     |                           |       |         |         |       |   |   |   |   |
| F.Muell. ex Cogn.               |                           |       |         |         |       |   |   |   |   |
| 3 N. Nicholson 3110 (BRI)       |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes tricuspidata*    | Lour. ssp. javanica       |       |         |         |       |   |   |   |   |
| Pruesapan & Duyfjes             |                           |       |         |         |       |   |   |   |   |
|                                 |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes tricuspidata*    |                             |       |         |         |       |   |   |   |   |
| Lour. ssp. tricuspidata         |                           |       |         |         |       |   |   |   |   |
| C.B.Clarke                      |                           |       |         |         |       |   |   |   |   |
| 1 P. Phonsena, W. de Wilde & B. Duyfjes 4414 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes truncata*        |                             |       |         |         |       |   |   |   |   |
| C.B.Clarke                      |                           |       |         |         |       |   |   |   |   |
| 2 P. Phonsena, W. de Wilde & B. Duyfjes 4490 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes truncata*        |                             |       |         |         |       |   |   |   |   |
| C.B.Clarke                      |                           |       |         |         |       |   |   |   |   |
| 3 P. Phonsena, W. de Wilde & B. Duyfjes 6329 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes villosa*         | Blume                     |       |         |         |       |   |   |   |   |
| 1 P. Phonsena, W. de Wilde & B. Duyfjes 4669 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes villosa*         | Blume                     |       |         |         |       |   |   |   |   |
| 2 P. Phonsena, W. de Wilde & B. Duyfjes 6331 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes villosa*         | Blume                     |       |         |         |       |   |   |   |   |
| 3 P. Phonsena, W. de Wilde & B. Duyfjes 4449 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes villosa*         | Blume                     |       |         |         |       |   |   |   |   |
| 4 P. Phonsena, W. de Wilde & B. Duyfjes 4000 (L) |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes villosa*         | Blume                     |       |         |         |       |   |   |   |   |
| 5 K. Pruesapan et al. 60 (L)   |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes fissibracteata*  |                           |       |         |         |       |   |   |   |   |
| C.Y.Wu ex C.Y.Cheng & Yueh      |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes wallichiana*     | (Ser.) Wight              |       |         |         |       |   |   |   |   |
| (Ser.) Wight                    |                           |       |         |         |       |   |   |   |   |
|                                 |                           |       |         |         |       |   |   |   |   |
| *Trichosanthes wawrae*          | Cogn.                     |       |         |         |       |   |   |   |   |
| B. Gravendeel et al. 631 (L)   |                           |       |         |         |       |   |   |   |   |
Polymerase chain reaction (PCR) amplification of purified total DNA was performed in 200 μl reaction tubes with a total volume of 50 μl. Each tube contained a mixture of 5 μl reaction buffer (ABgene, 10x), 3 μl MgCl2 (25 mM), 1 μl dNTPs (10 μM), 0.25 μl Taq-polymerase (ABgene; 5U/μl), 0.25 μl BSA (Roche Diagnostics), 12.5 μl of each primer (2 μM), 14.5 μl Milli-Q water and 1 μl template DNA. The ITS region was amplified using the primer pair ITS-P17 and ITS-26 S-82R [46] with the following PCR protocol 97°C 5 min., (97°C 30 s., 55°C 1 min., 72°C 1 min.) x 35, 72°C 10 min., 4°C ∞; matK with primers matK-2.1a [47] and matK-1440R [48], 95° 5 min., (95° 30 s., 50° 1 min., 72° 1 min.) x 35, 72° 10 min., 4° ∞; and rpl20-2.1a [48] and rps12 using the primers rpl20 and rps12 [49], 95° 5 min., (95° 30 s., 53° 1 min., 72° 1 min.) x 35, 72° 10 min., 4° ∞. Sequencing was performed using the primer pair ITS-P17 and ITS-26 S-82R [46] with the following PCR protocol 97°C 5 min., (97°C 30 s., 55°C 1 min., 72°C 1 min.) x 35, 72° 10 min., 4° ∞; a primer set consisting of 5 μl PCR product, 14.5 μl reaction buffer (ABgene, 10x), 3 μl MgCl2 (1.6 M), 0.25 μl BSA (Roche Diagnostics), and gap-coded using the Simmons and Ochoterena simulated annealing approach [31%], and latter regions increased statistical support values at early-branching clades. Sequences were concatenated, and gap-coded using the Simmonds and Ochoterena simple method [53] implemented in SeqState [54].

Sequence alignment
Sequence trace files were compiled into contigs with the program Gap4 and edited using Pregap4 [50], both part of the Staden package [51]. Sequences were aligned manually in Se-Al [52]. The final matrix included rpl20-rps12 (100% of taxa), ITS (96%), matK (84%), trnL-F spacer (31%), trnL intron (28%), and rbcL (20%). The three latter regions increased statistical support values at early-branching clades. Sequences were concatenated, and gap-coded using the Simmonds and Ochoterena simple method [53] implemented in SeqState [54].

Phylogenetic analyses
Selection of best-fit models of nucleotide substitution for the nuclear and plastid data partitions relied on the Akaike Information Criterion (AIC and AICc) as implemented in JModelTest version 0.1.1 [55,56]. Likelihood calculations were carried out for 88 substitution models on an ML-optimized tree. The best-fitting model for the combined data was the general time-reversible (GTR) model, with a proportion of invariable sites (I) and rate variation among sites (G) with four rate categories. Maximum likelihood tree searches and bootstrapping of the combined data (using 1000 replicates) relied on RAxML version 7.2.6 [57] on the CIPRES cluster [58].

Bayesian tree searching used MrBayes [59] on the CIPRES cluster [58]. The combined data were analyzed using three partitions (nuclear, plastid, gap data), allowing partition models to vary by unlinking gamma shapes, transition matrices, and proportions of invariable sites. Markov chain Monte Carlo (MCMC) runs started from independent random trees, were repeated twice, and extended for 10 million generations, with trees sampled every 1000th generation. We used the default priors in MrBayes, namely a flat Dirichlet prior for the relative nucleotide frequencies and rate parameters, a discrete uniform prior for topologies, and an exponential distribution (mean 1.0) for the gamma-shape parameter and branch lengths. Convergence was assessed by checking that the standard deviations of split frequencies were <0.01; that the log probabilities of the data given the parameter values fluctuated within narrow limits; that the convergence diagnostic (the potential scale reduction factor given by MrBayes) approached one; and by examining the plot provided by MrBayes of the generation number versus the log probability of the data. Trees saved prior to convergence were discarded as burn-in (10 000 trees) and a consensus tree was constructed from the remaining trees. The data matrix and trees have been deposited in TreeBASE (www.treebase.org; study number 12339).

Divergence time estimation
Divergence times were estimated using the Bayesian relaxed clock approach implemented in BEAST version 1.6.2 [60]. Searches used a Yule tree prior, the GTR+G substitution model, and 50 million MCMC generations, sampling every 1000th generation. Six monophyletic groups were defined based on the results of our phylogenetic analyses and previously published phylogenies [18,20,44]. Tracer version 1.5 [61] was used to check that effective sampling sizes had all reached >200, suggesting convergence of the chains. TreeAnnotator, part of the BEAST package, was then used to create a maximum clade credibility tree, with the mean divergence ages shown for all nodes with >95% highest posterior density.

Calibration relied on Cucurbitaceae fossils assigned to particular nodes (labeled A--C in Figure 3), using a gamma prior distribution with the fossil age as the offset and shape and scale parameter chosen to add a 95% CI of c. 10 Ma older than the fossil. (A) The root node, that is, the most recent common ancestor of Momordica and Trichosanthes, was constrained to 55.8 Ma with a shape parameter of 1.0 and a scale of 1.0, based on seeds from the Paleocene/Eocene Felpham flora representing the oldest Cucurbitaceae and dated to c. 55.8 Ma [62]. (B) The crown node of the Trichosanthes/Gymnopetalum clad was constrained to 34 Ma with a shape parameter of 1.0 and a scale of 3.4, based on Trichosanthes seeds from the Upper Eocene of Bulgaria [25] dated to c. 34 Ma and seeds from the Oligocene of West Siberia [26] dated to c. 23.8 Ma [27]. (C) The divergence of Marah and Echinocystis was set to 16 Ma with a shape parameter of 1.0 and a scale of 3.35, based on leaves and a fruit representing Marah from the Miocene of Stewart Valley, Nevada (M. Guilliams and D. M.
Erwin, University of California, Berkeley, in preparation; the fruit comes from the Fingerrock Wash site, dated to c. 16 Ma, the leaf from the Savage Canyon Formation, dated to c. 14.5 Ma). Absolute ages were taken from the geologic time scale of Walker and Geissman [63]. We also tested lognormal and exponential prior distributions, which gave very similar age estimates (not shown).

**Biogeographic analysis**

Biogeographic reconstruction relied on statistical dispersal-variance analysis using S-DIVA version 2.0 [64] as implemented in RASP, which carries out parsimony inference on the chain of trees obtained from an MCMC search [65,66], in our case the 8000 post burn-in Bayesian trees resulting from the BEAST dating analysis. S-DIVA averages the frequencies of an ancestral range at a node in ancestral reconstructions over all trees, with alternative ancestral ranges at a node weighted by the frequency of the node [64]. Range information for all species was compiled from taxonomic treatments [9,11,13-16], and the coded distribution areas were: A) Australia and New Guinea, B) Wallacea, C) Insular Sunda Malesia, D) Mainland Southeast Asia, E) India and adjacent countries, F) Africa, Europe and the New World.

**Authors’ contributions**

HB conceived the study, carried out the molecular genetic analyses, and drafted the manuscript. HS participated in the design of the study and data analysis, and also contributed field observations. SR and MT participated in the design and coordination of the study, and SR also helped with clock calibration and writing. All authors read and approved the final manuscript.

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