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Gisekia (Gisekiaceae): Phylogenetic relationships, biogeography, and ecophysiology of a poorly known C_4 lineage in the Caryophyllales[1]

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- *Premise of the study:* Gisekiaceae are a monogeneric family of the core Caryophyllales distributed in arid regions of Africa and Asia. The only widespread species of the genus, *Gisekia pharnaceoides*, performs C_4 photosynthesis based on CO_2 compensation point measurements. This study investigates the C_4 syndrome and its evolution in *Gisekia*. The infrageneric relationships, distribution and bioclimatic preferences of *Gisekia* are also investigated.
- *Methods:* Leaf gas exchange characteristics, activity of Rubisco and major C_4 cycle enzymes, and ultrastructural characteristics of mesophyll and bundle sheath cells are studied for *Gisekia pharnaceoides*. $\delta^{13}C$ values and leaf anatomy are analyzed for all species. A dated molecular phylogeny of 39 accessions representing all species of *Gisekiaceae* and 14 representatives of closely related core Caryophyllales families is generated using four cp markers and ITS. The precise current distribution and bioclimatic niche of *Gisekia* is assessed on the basis of 520 georeferenced specimen localities.
- *Key results:* All traditionally recognized species of *Gisekia* are C_4 plants with atriplicoid Kranz anatomy. *Gisekia pharnaceoides* uses the NAD-ME biochemical type. The molecular phylogeny demonstrated two East African clades nested within South African clades, demonstrating migration along the arid areas of eastern Africa during the Late Miocene/Pliocene Epochs. Most traditionally defined species are polyphyletic.
- *Conclusions: Gisekia* represents an isolated C_4 lineage within core Caryophyllales dating back to the Miocene Epoch and probably spread along the African arid corridor from a South African center of origin. The seven currently recognized species should be treated as one polymorphic species or species complex, *Gisekia pharnaceoides* agg.

Key words: arid corridor; Africa; C_4 photosynthesis; carbon isotope values; *Gisekia*; Gisekiaceae; Kranz anatomy; phylogeny.

The rise of C_4 photosynthesis is one of the outstanding events in the recent history of the biosphere. Over the past 35 million years, the C_4 pathway has independently evolved in over 65 lineages of angiosperms, making it one of the most fascinating examples of convergent evolutionary change on Earth (R. Sage et al., 2011, 2012). Since their origin, C_4 plants have given rise to the expansive grasslands, savannas and shrublands from the tropics to the warm temperate zone, which has triggered widespread diversification of grassland adapted animal guilds, one of which is likely our own species, *Homo sapiens* (Edwards et al., 2010). How and why the C_4 pathway evolved remain important questions and are best addressed by studies that compare evolutionary transitions from C_3 to C_4 species (Ackerly, 1999). This comparative approach treats independent lineages as evolutionary replicates, and thus the more than 65 independent lineages of C_4 photosynthesis could provide many replicates to address hypotheses regarding C_4 evolution and its consequences. However, many lineages are unsuitable for studying how and why the C_4 pathway evolved, due to a loss of intermediate forms and extensive change since the origin of the pathway within a lineage. Younger lineages, in particular those with few species, often prove to be of greater value as they still retain intermediate forms and vestiges of their early C_4 state. For example, *Flaveria* (Asteraceae) has only 22 species, but contains a range of forms from full C_3 to full C_4 and nine C_3-C_4 intermediates with varying degrees of C_4-ness (Ku et al., 1991; McKown et al., 2005). For this reason, *Flaveria* has been the principle model for C_4 evolution, but it stands alone as all other groups have a limited number of C_3-C_4 species and often relatively poor phylogenetic coverage. Only *Heliotropium* (Boraginaceae), with five C_3-C_4 intermediate species, has more than 2 intermediates in a sister position to a C_4 clade (Muhaidat et al., 2011). Thus, there is a need to identify new lineages of recently evolved C_4 groups to increase the number of useful replicates available to address questions of C_4 evolution. One potentially valuable group that has received little attention is *Gisekia*, a small, largely African genus of seven species known to contain at least one C_4 species, *Gisekia pharnaceoides* L. (Rama Das and Raghavendra, 1972).

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The Gisekiaceae is a monogeneric family of uncertain position within the core Caryophyllales. According to recent molecular studies, the family is closely related to Aizoaceae, Agdestidaceae, Nyctaginaceae, Phytolaccaceae, and Sarcobataceae (Brockington et al., 2009; Schäferhoff et al., 2009; Crawley and Hilu, 2012). Although excluded from Aizoaceae and raised to family level in 1942 by Nakai (Nakai, 1942), *Gisekia* has since been affiliated with three different families. It has been classified back into Aizoaceae (Hauman, 1951; Adamson, 1961), and Molluginaceae (Andrews, 1950; Friedrich, 1959; Eckardt, 1964), while Hofmann (1973) considered it as closely related to Phytolaccaceae (for summary see Gilbert, 1993). Only after the revision by Gilbert (1993) has the family rank of Gisekiaceae been accepted.

According to the taxonomic revision by Gilbert (1993) *Gisekia* consists of seven species, *G. pharnaceoides* L., *G. diffusa* M. Gilbert, *G. paniculata* Hauman, *G. africana* (Lour.) Kuntze, *G. haudica* M. Gilbert, *G. scabridula* M. Gilbert, and *G. polylopha* M. Gilbert (Table 1). Within *G. pharnaceoides,* there are two varieties, var. *pharnaceoides* L. and var. *alata* M. Gilbert, and within *G. africana* there are three: var. *africana* (Lour.) Kuntze, var. *pedunculata* (Oliv.) Brenan, and var. *decagyna* Hauman (Gilbert, 1993). The morphological basis for species rank is a set of diagnostic character states, with stamen number, mericarp surface structure, and pedicel length being the most important ones (Table 1).

Gisekia species are mostly prostrate annuals (rarely short-lived perennials persisting over a few years) with linear to lanceolate, flat leaves (Appendix S1; see supplemental figure with the online version of this article). The genus typically grows in open habitats in sandy or stony soils. It shows a scattered distribution throughout Africa, with most species occurring in East Africa (Gilbert, 1993; Table 1). Only the widely distributed ruderal *G. pharnaceoides* occurs outside Africa, notably in Asia (Arabian Peninsula, Iran, Pakistan, India, Myanmar, Thailand, Vietnam) and as an introduced weed in North America (Florida) (Godfrey, 1961). In their physiological study of the dicotyledonous weed flora in India, Rama Das and Raghavendra (1972) observed a CO_2 compensation point typical of C_4 photosynthesis for *G. pharnaceoides*. This is the only published evidence for C_4 photosynthesis in *Gisekia* up to this date.

Here we investigate infrageneric relationships and placement of Gisekiaceae within the order Caryophyllales using sequence variation of five molecular markers. We then determine the photosynthetic pathway of all species in the genus and characterize the biochemical and structural attributes of *G. pharnaceoides*. Finally, we characterize the distribution and bioclimatic niche of the genus, to address where the C_4 pathway may have

TABLE 1. Species of *Gisekia* after Gilbert[a] their morphology, distribution and sampling in this study.

Species and distribution	General morphological differences	Mericarp morphology	Notes	Sampling
		5 stamens		
***G. diffusa* M.Gilbert** Ethiopia, Kenya, Somalia	Annual herb, ± upright growth, linear leaves, terminal cymes, fruiting pedicels thin, straight and long (10–14 mm).	With spines.	Included in *G. pharnaceoides* prior Gilbert (1993).	Gise 11, 12, 13, 27, 28, 29, 30
***G. paniculata* Haumann** Zaire	As *G. diffusa*, only difference fruiting pedicels straight and short (1–6 mm).	With spines.	Included in *G. pharnaceoides* prior Gilbert (1993).	Gise 10
***G. pharnaceoides* L.** Africa, Madagascar, Mascarene Islands, South Asia	Annual herb, prostrate growth only rarely ascending, elliptic to linear leaves, umbels, fruiting pedicels bend, 3–8 mm long.	Smooth to spiny papillose or obscurely toothed (var. *pharnaceoides*). Smooth to obscurely papillose with prominent whitish crests or wings (var. *alata*).	Type species of *Gisekia.*	Gise 2, 3, 4, 5, 19, 21, 31, 36, 38, 39, 40, 46, 47, 48
		10-15 stamens		
***G. africana* (Lour.) Kuntze** East Africa, South Africa to Mozambique	Annual herb, habit variable but not prostrate, linear leaves, lax terminal cymes.	Papillose to spiny (var. *africana* and var. *decagyna*). Prominent, pale dorsiventral wing (var. *pedunculata*).		Gise 6, 24, 25, 26, 33, 34, 35, 37, 41, 43,
***G. haudica* M.Gilbert** Ethiopia, Somalia	Annual, reddish herb, prostrate growth, rectangular-linear leaves, dense axillary umbels.	Papillose to spiny, dorsiventral wings, sometimes lateral wings.	Included in *G. africana* prior Gilbert (1993).	Gise 17, 18
***G. polylopha* M.Gilbert** Somalia	Annual herb, ascending growth, with minute papillae at nodes, lax dichasial cymes.	Lateral triangular teeth or wings, somtimes reticulately arranged, broad sutural wings.		Gise 14, 15, 16
***G. scrabidula* M.Gilbert** Somalia	Perennial herb, prostrate growth, with scales on stems and leaves, linear leaves, axillary, lax umbels, pedicels straight or curved.	Lateral triangular teeth or crests, sometimes joined into wings.		Gise 7, 51, 52

[a] Gilbert (1993).

originated, and what the potential climatic drivers of its evolution may have been.

MATERIALS AND METHODS

Material—For the phylogenetic, morphological, anatomical and bioclimatic study and for the carbon isotope measurements, we used specimens from the following herbaria: BR, BOL, C, E, HBG, EA, K, UPS and W, or samples conserved in 70% ethanol collected by one of us (H. E. K. Hartmann). For the physiological and biochemical characterizations, we collected *G. pharnaceoides* seeds on the outskirts of Orlando, Florida, USA, from a disturbed sandy roadside among orange groves. Plants were common at the collection site (Appendix S1; Highway 545, 5.0 miles N of the junction of the intersection of Highways 545 and 530; 28°24′N × 81°38′W—vouchers deposited in the Royal Ontario Museum (ROM) herbarium, Toronto, Canada). Experimental plants were germinated from seed in a sandy-loam mix and grown in a plant growth chamber at 28°C days/22°20°C nights and a photon flux density of 500 μmol m^{-2} s^{-1}. All accessions included in this study are listed in the online supplementary material (Appendix S2; see supplemental table with the online version of this article).

Geographical and bioclimatic analysis—The distribution area of the family was assessed using 520 herbarium specimens, 260 of which were only available as digital pictures. Coordinates of locations for all herbaria accessions were either taken directly from the information given on the herbarium sheet or reconstructed using collection site information (e.g., cities, distances, altitude) and Google Earth Pro. (Google Inc., Mountain View, CA USA). All accessions were embedded into DIVA-GIS version 7.4.0.1 (Hijmans et al., 2005) for analysis of distribution and climatic niche (according to the 19 climatic variables implemented in DIVA-GIS). In addition, each distribution and the climatic niches of the clades found in the phylogenetic analyses were analyzed.

Phylogenetic inference—55 accessions were used for DNA extraction for the phylogenetic analysis. These include seven accessions to be used as outgroup species (representatives of *Alluaudia*, *Bougainvillea*, *Claytonia*, *Didieria*, *Hypertelis*, *Minuartia*, *Rhipsalis*) and 48 accessions of *Gisekia* (*G. africana* (11), *G. diffusa* (6), *G. haudica* (2), *G. pharnaceoides* (21), *G. polylopha* (2), *G. paniculata* (2), *G. scrabidula* (4)) that were used as ingroup species.

Furthermore, we included 26 sequences deposited in GenBank—mainly of other families in the core Caryophyllales. These were selected according to the results of recent orderwide molecular studies (Brockington et al., 2009; Schäferhoff et al., 2009; Crawley and Hilu, 2012). Total DNA was extracted from 20 mg dried leaf-material using the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's specifications. PCR was carried out in T-Professional or T-Gradient Thermocycler (Biometra, Göttingen, Germany). We amplified the *atpB-rbcL* spacer and ITS as described in Kadereit et al. (2006) while the amplification of *petB-petD* spacer and *matK* gene was performed after Schäferhoff et al. (2009). The amplification of the *rpL*16 intron was carried out following Shaw et al. (2005). For ITS the primers ITS-28S and ITS-18S (both Kadereit et al., 2006), for the *petB-petD* spacer Pi*petB*-1411F and Pi*petD*-738R (both Löhne and Borsch, 2005), for the *matK* gene TrnKF-bryo (Wicke and Quandt, 2009) and AC*matK*1401R (Schäferhoff et al., 2009), for the *rpL*16 intron *rpL*16-F71 and *rpL*16-R1516 (both Small et al., 1998), and for the *atpB-rbcL* spacer *atpB-rbcL*-F and *atpB-rbcL*-R (both Xu et al., 2000) were used. PCR products were checked on 0.8% agarose gels and purified subsequently using 0.4 μL ExoSAP-IT PCR Clean-up (Affymetrix, Santa Clara, California, USA) following the manufacturer's manual. DNA sequences were obtained using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) in combination with the primers just mentioned following a purification step using IllustraTM SephadexTM G-50 Fine DNA Grade (GE Healthcare, Munich, Germany). DNA fragments were sequenced via the automatic capillary sequencer GA3130XL (Applied Biosystems, Foster City, California, USA) following the Sanger method.

Forward and reverse sequences were edited and merged to consensus sequences, which then were aligned using Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and Mesquite version 2.75 (available at http://mesquiteproject.org). All alignments were checked and corrected manually applying the similarity criterion (Simmons, 2004). Ambiguously aligned nucleotide positions were excluded from the analyses. Gaps were treated as missing data. Two analyses were conducted: (1) All cp data with a broad sampling of closely related families in the core Caryophyllales and *Minuartia laricifolia* (Caryophyllaceae) as the outgroup, and (2) ITS sequence data for *Gisekia* with two members of the Aizoaceae family as the outgroup. ITS was not included in the first matrix because it is too variable at intrafamilial level in Caryophyllales and because of some incongruencies between cp and ITS tree topologies. A combination of cp data and ITS data only for samples of *Gisekia* shows the cp tree topology with slightly different support values (not shown). The alignments of the cp data and ITS data are available as supplemental data with the online version of this article (Appendix S3 (cp data) and Appendix S4 (ITS)).

For both data sets maximum likelihood (ML) (Felsenstein, 1973) trees were calculated with RAxML (Stamatakis et al., 2008) using 100 rapid bootstrap inferences and in the GTR+G substitution matrix. For the cp data set phylogenetic trees based on a Bayesian Markov Chain Monte Carlo (MCMC) approach (Yang and Rannala, 1997) were generated using Bayesian Evolutionary Analysis by Sampling Trees (BEAST) version 1.5.4 (Rambaut and Drummond, 2013; Drummond and Rambaut, 2007). The BEAST xml input files (available as an online supplement) were created with BEAUti v1.5.4 (Drummond and Rambaut, 2007). The following settings were used: substitution model GTR + G with four categories for G, relaxed clock model with a lognormal distribution, birth and death demographic model, MCMC with 20 000 000 iterations initiated and performed with sampling frequency of 1000. We used the estimated age 56.04 my (sd = 4.70) of the common ancestor of the clade comprising Molluginaceae to Nyctaginaceae from Christin et al. (2011b: fig. 2) to constrain the age of the ingroup. Topological convergence of two independent runs was confirmed using TRACER (Rambaut and Drummond, 2013). A burn-in of 1000 trees was discarded. Posterior probability (PP) clade support was calculated together with the medians and 95% confidence limits for ages of the nodes.

Morphological observations—All accessions were identified using the key of Gilbert (1993). The diagnostic characters for species delimitation described in Gilbert´s revision were carefully examined for 278 specimens received on loan. In addition, the mericarp structures of the accessions used in the phylogenetic analysis were analyzed using an XL 30 ESEM (Philips) for electron microscopy.

Leaf anatomical studies—Fresh material was only available for the one accession of *G. pharnaceoides* from Florida. Leaf samples from these plants were imaged using light and transmission electron microscopy following fixation in 1.5% glutaraldehyde and embedding in Spurrs resin, as previously described (Sage and Williams, 1995). To investigate leaf anatomical variation among species of *Gisekia,* we prepared samples from herbarium specimens by rehydrating leaves for 72 h in a 10% NH_3 solution. Samples were then dehydrated and in a sequence of ethanol solutions (15%, 30%, 50%, 70%, 80%, 90%, 96% ethanol) and finally 96% ethanol and isobutanol 1 : 1 solution (each step 30 min). The samples were then placed in pure isobutanol for 48–72 h. The 80% ethanol solution contained two spatula points Eosin Y per 200 ml to stain the samples with a faint red and improve the infiltration of paraffin. Melted paraffin was added to the samples in isobutanol (mixture 1 : 1) and incubated for 48 h at 60°C. After all isobutanol had evaporated, samples were transferred into small paraffin blocks. Optimal sections were 14 μm thick. Sections were dried and carefully bathed for 45 s in a toluidine blue bath for staining and then washed in distilled water bath, after drying at 40°C they were bathed in Roti-Histol for 20 min to wash away the paraffin. Sections were studied with a light microscope (Diaplan, Leica Microsystems, Wetzlar, Germany) and documented with an attached camera (DFC 420C, Leica Camera, Solms, Germany). The following samples were investigated: *G. africana* var. *africana* (V-539581, V-539535, V-538459), *G. africana* var. *decagyna* (V-539591), *G. africana* var. *pedunculata* (V-539529), *G. haudica* (V-539534), *G. pharnaceoides* var. *pharnaceoides* (V-539603, V-539624, V-130864, V-539615), *G. pharnaceoides* var. *alata* (V-539605), *G. polylopha* (V-051235), *G. scabridula* (V-048631; all from UPS, see Appendix S2).

Measurement of $\delta^{13}C$ values—The carbon isotope ratio of 1–2 mg samples from adult leaves or stems of 46 specimens was measured using isotope ratio mass spectrometry (Appendix S5; see supplemental table in the online version of this article; Smith and Epstein, 1971). Samples were analyzed with a mass spectrometer either by the University of California, Davis isotope facility (http://stableisotopefacility.ucdavis.edu), or at the Institut für Geowissenschaften, University of Mainz, Germany.

Gas Exchange and Biochemistry—The response of net CO_2 assimilation rate (*A*) was measured using a LiCor Li-6400 gas exchange system (Li-Cor, Lincoln, Nebraska, USA) at 30°C. Leaves were equilibrated in the leaf cuvette at the current atmospheric CO_2 conditions and then the ambient CO_2 was doubled to 1000 μmol mol^{-1}. After leaf gas exchange equilibrated to this CO_2 level (about 15 min), measurements commenced using the Li-6400 auto-program. The CO_2 content in the leaf chamber was then decreased in a series of 10 steps, with measurements being conducted at each CO_2 level after a 15 min equilibration period.

The activity of Rubisco and the major C_4 enzymes in *G. pharnaceoides* was determined using the methods of T. Sage et al. (2011). In brief, following extraction in a bicine buffer, enzyme activities were assayed with a diode array spectrophotometer by coupling the enzyme activity to the oxidation or reduction of NAD(P)$^+$ or NAD(P)H, respectively.

RESULTS

Geographical and bioclimatic analysis—The bioclimatic envelope of *Gisekia* based on 538 georeferenced data points shows that the genus occurs in regions with 14–30°C mean annual temperature and 46–794 mm mean annual precipitation (Fig. 1). It has been collected between 0–2500 m altitude; however, it generally occurs below 500 m. Information collected from herbarium labels indicates that the genus mostly grows on sandy substrate or gravel, often in ruderal, disturbed and open vegetation. It often occurs in or near seasonally dry lakes and river beds (Fig. 1, inset).

Members of *Gisekia* are most common in southern Africa, East Africa, and on the Arabian Peninsula (Fig. 1). In Africa, there is no evidence for its occurrence in the following countries: Cameroon, Guinea, Gabon, Central African Republic, Cote D'Ivoire, Benin, Western Sahara, Libya and Egypt. In Asia it is absent from Qatar, Kuwait, Afghanistan, Nepal, Laos, and Cambodia. The species recognized by Gilbert (1993) overlap in their distribution (Fig. 1) and the comparison of individual bioclimatic niches for the species with DIVA-GIS shows that they share similar climatic regions (data not shown).

Phylogenetic inference and dating—Using 38 accessions of all seven species of *Gisekia* (Table 1) and 14 species from related families, we generated a dated tree based on 3254 aligned nucleotides from four different cp regions (*atpB-rbcL* spacer, *petB-petD* spacer, *matK* gene, *rpL*16 intron). The family Gisekiaceae is monophyletic with high statistical support (bootstrap 100, pp 1; Fig. 2). The closest relative of the Gisekiaceae remains unclear. The family forms an unsupported sister relationship with a clade consisting of Phytolaccaceae, Nyctaginaceae and Aizoaceae. This clade plus Gisekiaceae are sister to *Hypertelis* (formerly part of Molluginaceae, which will probably be classified as a family of its own; Fig. 2). The stem of Gisekiaceae is dated at 37.1 mya (95% confidence interval: 49.4–22.9) and the extant species in the family started to diversify 14.9 mya (95% confidence interval: 25.7–6.2 mya) (Fig. 2). The ITS tree topology is largely unresolved. However, it supports the basal grade found in the cp data and some internal branches (Appendix S6; see supplemental figure in the online version of this article). On the other hand there are also some accessions with supported conflicting positions in the two trees (marked orange in Appendix S6). Both gene trees clearly show that all species recognized by Gilbert (1993), except for *G. paniculata*, which was represented by one accession only, are polyphyletic. Instead of a taxonomic signal, a geographical signal is apparent in the cp tree with three South African and two East African lineages (Fig. 2).

Morphological observations—Species identification using the key provided by Gilbert (1993) was possible for most accessions (Appendix S2), indicating the existence of distinct morphotypes (Table 1). However, the morphotypes with a 10–15 staminate androeceum are present in multiple clades according to the molecular results (Fig. 2). The mericarp surface is highly variable in *Gisekia*. The outgrowths can be papillate, with narrow or broad teeth, regular or irregular crests and small, or large wings with irregular margins (Fig. 3). Mostly there are two or more different outgrowth structures on one mericarp (Fig. 3B-F). Mericarps of one flower normally show the same types of outgrowth, albeit these might vary in their proportions and distribution on the surface. This polymorphism makes mericarp structure a difficult diagnostic feature in *Gisekia*.

C_4 anatomy and ultrastructure—Leaf cross sections from cultivated plants of the Florida accession showed that *G. pharnaceoides* has a typical atriplicoid type of Kranz anatomy, with bundle sheath (BS) tissue surrounded by a single row of mesophyll (M) cells (Fig. 4). Adaxial mesophyll cells are elongated while central and abaxial mesophyll cells are more rounded, thus forming a bifacial leaf anatomy (Fig. 4B). Mesophyll and BS cells are of similar size in cross section (Table 2). A layer of hypodermal water storage cells lines the bottom of the leaf between the Kranz units and the epidermis. Also, the epidermal cells on both sides of the leaf are slightly succulent. These cells (the epidermis and subepidermal layer on the lower side) contribute to the succulent nature of the linear leaves. Stomata occur on both sides of the leaf directly above pronounced intercellular air spaces.

Many chloroplasts are densely packed into the inner, centripetal regions of the BS cells, while M chloroplasts are distributed around the M cell periphery (Fig. 4A–C). A BS cell contains three times as many chloroplasts as an M cell although the BS and M chloroplasts are identical in size. As a result, chloroplasts cover 2.7 times more of the BS tissue in cross-section than does M tissue (Table 2). Chloroplasts cover about half of the M cell periphery, which is below the norm for C_3 species in which chloroplasts cover 80% or more of the cell periphery opposite the intercellular spaces (Evans and Loreto, 2000; Busch et al., 2013). Chloroplasts in the BS cells are typically elongated cylinders, allowing for tighter packing (Fig. 4B,D).

Mitochondria are over five times more numerous in the BS than in the M cells (Table 2). In the BS, the mitochondria are located in the inner half, mostly along the cell periphery; only a few BS mitochondria are sandwiched between chloroplasts (Fig. 4D). Large numbers of plasmodesmata are visible in the wall connecting BS and M cells (Fig. 4G). Chloroplasts of BS cells have greater stacking of thylakoids than M cell chloroplasts, as indicated by a twofold greater granal index (Table 2; Fig. 4E,F,I).

Sections from dried leaf material of six of the seven species recognized by Gilbert (1993; see Appendix S2 for the specimens used for anatomical studies) also show typical atriplicoid anatomy as described previously (not shown). Overall, there is little variation among the samples. In one sample (*G. africana* var. *africana* (UPS: V539581)) the mesophyll cells are elongated above and below the vascular bundles. Furthermore, leaves vary in the amount of idioblasts containing raphides (Appendix S7; supplemental figure in the online version of this article). The epidermal cells vary in size in some samples with the cells of the lower epidermis being distinctly larger than those of the upper epidermis (e.g., *G. haudica* (UPS: V539534) and *G. pharnaceoides* var. *pharnaceoides*

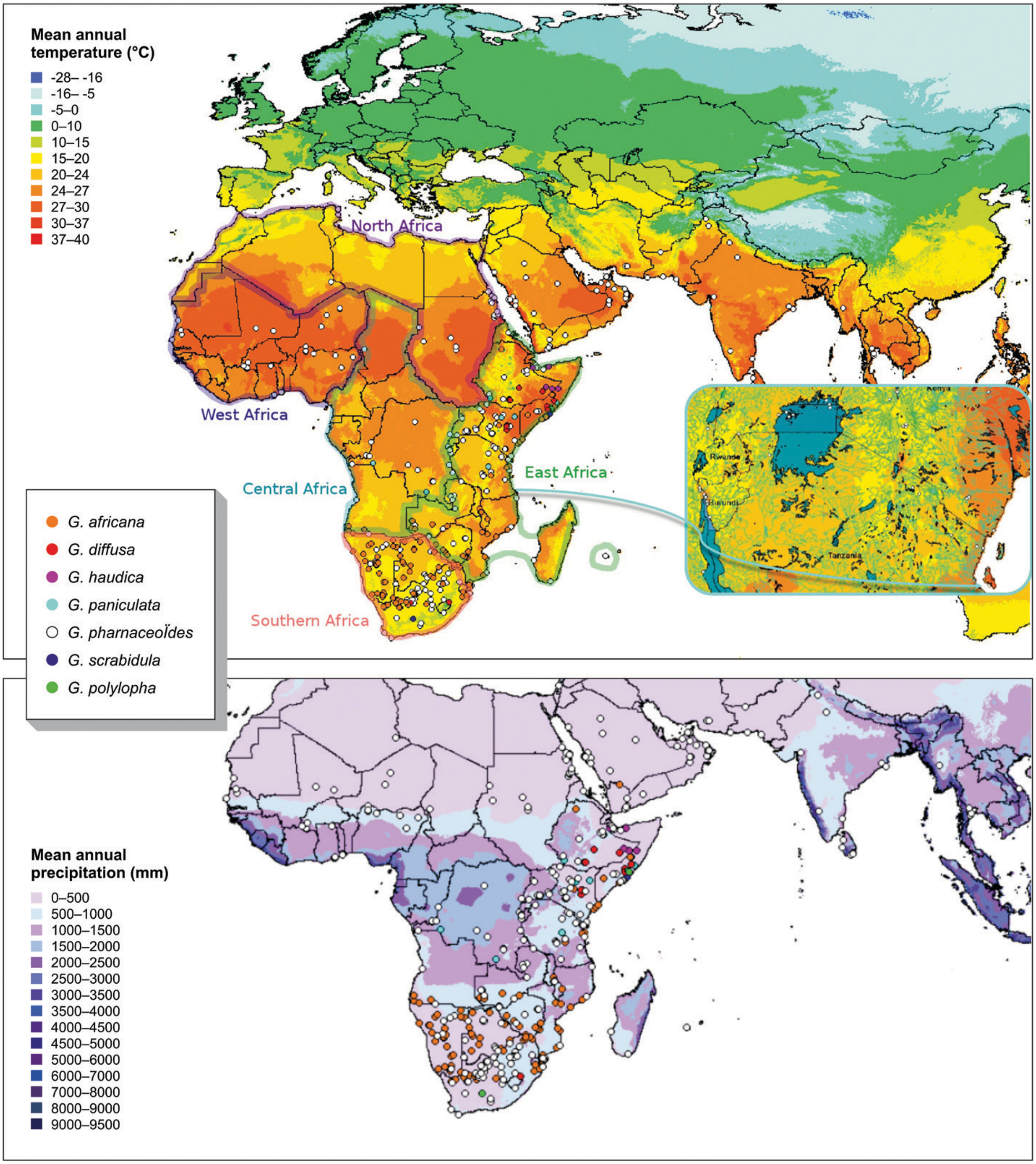

Fig. 1. Distribution of *Gisekia* (Gisekiaceae) in Africa and South Asia based on 538 georeferenced data points (Appendix S2 for voucher information).

(UPS: V539605)). Some samples of *G. scrabidula* and *G. pharnaceoides* show distinct triangular protrusions on the midvein (Appendix S7).

δ¹³C values, gas exchange and biochemistry—Carbon isotope values of 46 accessions representing six species of *Gisekia* range between −9.2 ‰ and −16.9 ‰, which demonstrates that all

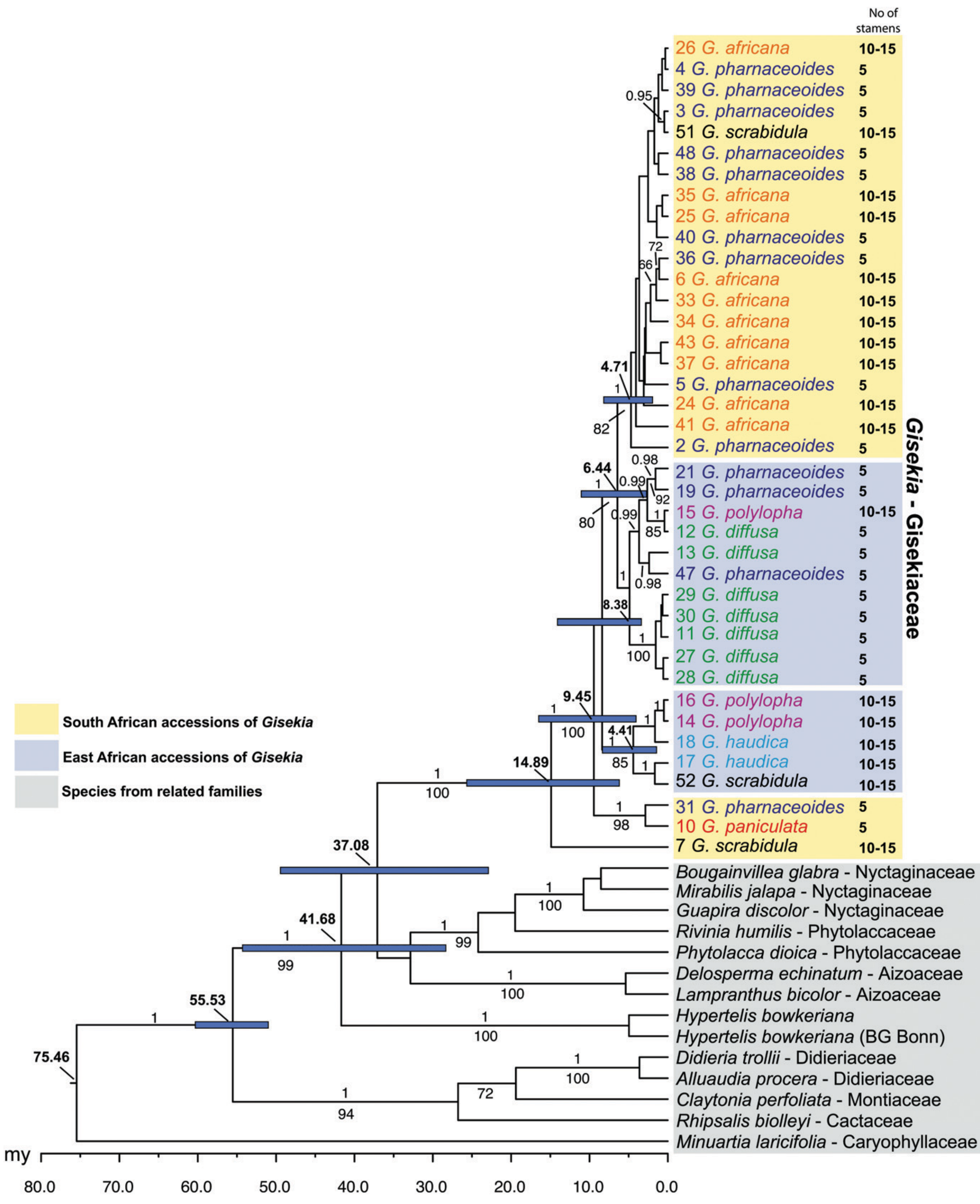

Fig. 2. Dated molecular phylogeny of Gisekiaceae showing its position among closely related families of Caryophyllales. The tree is based on 3254 aligned bp gained from four cp markers and Bayesian inference using BEAST. Bold numbers along branches represent node ages, regular numbers above branches represent posterior probabilities > 0.94, regular numbers below branches are maximum likelihood (ML) bootstrap values > 65 and number in terminals represent laboratory codes. The node bars represent 95% confidence intervals of node ages.

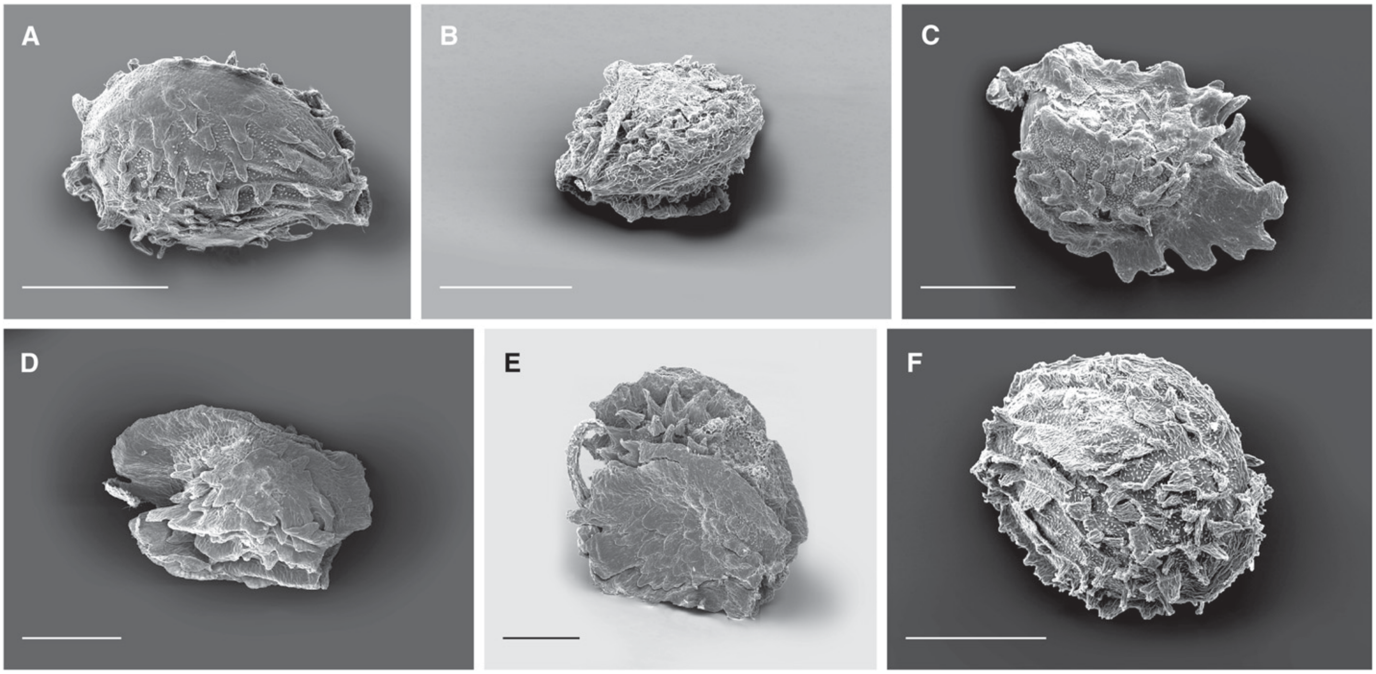

Fig. 3. Mericarp surface structures in different species of *Gisekia*. (A) *G. diffusa* (Gise 11; Friis, Tadesse & Vollesen 2940, UPS), (B) *G. haudica* (Gise 18; Thulin & Warfa 5365, UPS), (C) *G. africana* (Gise 15; Friis, Gilbert & Vollesen 3666, UPS), (D) *G. polylopha* (Gise 14; Thulin, Hedrén & Dahir 7318, UPS), (E) *G. pharnaceoides* (Gise 40; Skarpe 364, UPS), (F) *G. scrabidula* (Gise 52; Thulin & Dahir 6432, UPS). Scale bar = 500 µm. (For full voucher information, see Appendix S2.)

Gisekia species use the C_4 photosynthetic pathway (Appendix S5; Cerling, 1999).

The photosynthetic capacity of *G. pharnaceoides* is typical of a weedy C_4 species, ranging between 30 and 45 µmol m^{-2} s^{-1} at 30°C (Fig. 5). The response of the net CO_2 assimilation rate to intercellular CO_2 content (the A/C_i response) demonstrates that *Gisekia pharnaceoides* has a near typical photosynthetic behavior for a C_4 plant (Fig. 5). The CO_2 compensation point is near 0 µbar, the initial slope of the A/C_i response is steep, indicating a high carboxylation efficiency, and there is a relatively sharp transition from the initial slope to a nearly CO_2 saturated rate of photosynthesis at a C_i below 100 µbar (Table 3). The only deviation from a typical C_4-type of response is a drift upwards in the A value above the transition to near CO_2 saturation. C_4 plants typically have a flat response of A above the sharp transition to CO_2 saturation; a drifting upwards of A above this point indicates that there may be some residual activity of Rubisco and C_3 photosynthesis outside the BS cells, possibly in the chloroplasts of the water storage tissue (Khoshravesh et al., 2012).

Assay of the activity of Rubisco, phosphoenolpyruvate (PEP) carboxylase, and the three possible C_4 decarboxylating enzymes further demonstrate that *G. pharnaceoides* is a C_4 plant (Table 3). Rubisco activity in vitro is close to the observed value of A at 30°C, as is typical for C_4 species; in C_3 species, Rubisco activity is typically 3 to 4 times the observed A (Sage, 2002). PEP carboxylase activity is nearly 5 times the value of Rubisco activity which is also typical for C_4 species. Of the decarboxylating enzymes, NAD-malic enzyme exhibited activity that is similar to observed values of A (Table 3), while the NADP-ME and PEP carboxykinase activities are very low and similar to values observed in C_3 species (Muhaidat et al., 2011) demonstrating that *G. pharnaceoides* uses the NAD-ME subtype of C_4 photosynthesis.

DISCUSSION

The monogeneric family Gisekiaceae belongs to the raphide clade of the core Caryophyllales (Hilu et al., 2003; Brockington et al., 2009; Schäferhoff et al., 2009; this study: Fig. 2). The molecular studies consistently show that *Gisekia* is not particularly close to any other family of the raphide clade but originates from a common lineage together with Aizoaceae, a clade comprising Phytolaccaceae, Nyctaginaceae, Agdestidaceae and Sarcobataceae, the genus *Hypertelis* (formerly Molluginaceae) and *Lophiocarpus* (Lophiocarpaceae). This scenario supports the hypothesis of Nakai (1942) that *Gisekia* should be classified as a family of its own, the Gisekiaceae.

***The C_4 syndrome in* Gisekia—**All members of *Gisekia* and Gisekiaceae are C_4, as shown by the isotopic data and the light and TEM images (Appendix S5; Figs. 4 and 5). Anatomically, *Gisekia* develops the atriplicoid version of Kranz anatomy, which is the most common form in the eudicots (R. Sage et al., 2011). With *Gisekia*, the atriplicoid anatomy is now known to occur in nearly 60% of the eudicot C_4 lineages. *Gisekia pharnaceoides* utilizes the NAD-ME subtype of C_4 metabolism, which occurs in a third of the C_4 species for which the subtype is known (R. Sage et al., 2011). In the Caryophyllales, 11 of 23 (48%) of the C_4 lineages possess the NAD-ME subtype, indicating that members of this order are more likely to have evolved NAD-ME as the principle decarboxylating enzyme than are other orders of the plant kingdom. A comparative study of *Gisekia* and the other NAD-ME Caryophyllales may provide clues as to why this subtype was preferred during C_4 evolution.

In *Gisekia*, the bundle sheath chloroplasts exist as elongated cylinders that are packed closely together in the inner half of the bundle sheath cells (Fig. 4). Eudicot NAD-ME species generally

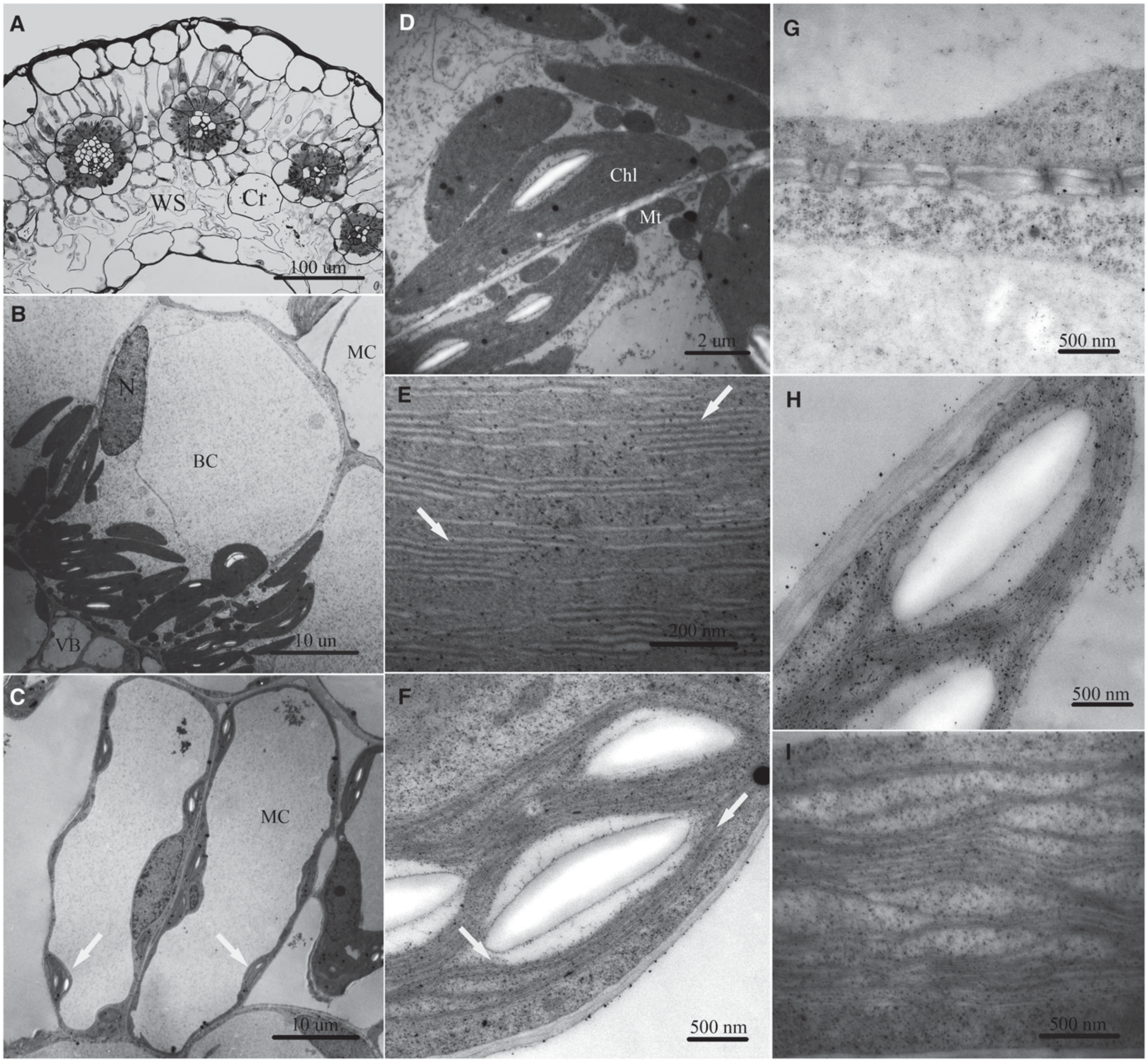

Fig. 4. (A) Light microscopy of a cross section of *Gisekia pharnaceoides* leaf. B–I, Transmission electron micrographs: (B) An individual bundle sheath cell; (C) Mesophyll cells; (D) High magnification of bundle sheath cross section shows several mitochondria in contact with chloroplasts; (E) High magnification of bundle sheath chloroplast, arrows show appressed grana; (F and I) High magnification of mesophyll cell chloroplast, arrows show nonappressed grana; (G) Plasmodesmata connecting mesophyll and bundle sheath cells; (H) Chloroplast from water storage cells. *Figure abbreviations*: BC, bundle sheath cell; Chl, chloroplast; Cr, crystal; MC, mesophyll cell; Mt, mitochondria; VB, vascular bundle; WS, Water storage cell.

exhibit this shape and packing arrangement of the bundle sheath chloroplasts (Black and Mollenhauer, 1971; Dengler and Nelson, 1999; Marshall et al., 2007; Voznesenskaya et al., 2010), and it is common for mitochondria to be positioned along the lengths of the chloroplasts. NAD-ME is located in the BS mitochondria, so this close arrangement of chloroplast and mitochondria facilitates rapid uptake of the CO_2 released by NAD-ME, and reduces CO_2 leakage from the BS. The high NAD-ME activity also explains why the number of mitochondria in the BS is over five times that of the M cells (Dengler and Nelson, 1999). In BS cells of *G. pharnaceoides*, many mitochondria are sandwiched between the elongated chloroplasts and the cell wall. This pattern is common in C_3–C_4 intermediate species using a glycine shuttle to concentrate CO_2 in the BS tissues (Sage et al., 2012). Its presence in *G. pharnaceoides* suggests that *Gisekia* retains vestiges of the intermediate state and may not have a fully optimized C_4 mechanism. Further support for a less optimal C_4 mechanism is evident in the A/C_i analyses that show a slight increase in A with increasing C_i above the initial slope region of the A/Ci response (Fig. 5). Optimized C_4 species exhibit a flat

Table 2. Properties of cells and organelles in leaves of *Gisekia pharnaceoides*.

Cell type	Bundle sheath	Mesophyll
Cell area (μm^2)	1710 ±164	1795 ±278
Chloroplasts per cell area × 10^3 µm	10.8 ±0.8[a]	4.2 ± 0.7[b]
Area per chloroplast (μm^2)	26.0 ±3.7	24.3 ±3.4
Total chloroplast area per cell area, %	26.6 ±2.7[a]	9.6 ± 1.4[b]
Mitochondria per cell area × 10^3 µm	20.8 ±2.9[a]	4.0 ±0.9[b]
Total mitochondria area per cell area, %	2.2 ±0.2[a]	0.3 ±0.1[b]
Area per mitochondrion (μm^2)	1.1 ±0.1[a]	0.9 ±0.1[b]
Granal index (%)[†]	47 ± 2[a]	22 ±3[b]
Granal density (μm^{-1})[‡]	17 ±2	18 ±3

Note: N = 5 plants. Standard error is given after mean. Ten cells were measured per plant, and averaged to produce a plant value. Superscripted letters indicate statistical differences between bundle sheath and mesophyll values at $p < 0.05$, using a Student's *t* test with plant means as the unit of replication.

[†] Granal index is calculated by dividing the length of appressed grana to the length of total (appressed + nonappressed) chloroplast grana.

[‡] Granal density is measured as a ratio of total chloroplast grana (µm) to chloroplast area (μm^2).

Table 3. Summary of leaf gas exchange and photosynthetic enzyme activities for *Gisekia pharnaceoides*.

Parameter	Mean ±SE
Leaf Gas Exchange[1]	
Net CO_2 assimilation rate	33.7 ± 5.1 µmol m^{-2} s^{-1}
Stomatal conductance	0.30 ± 0.06 mol m^{-2} s^{-1}
C_i/C_a[3]	0.49 ± 0.02
Water use efficiency	6.00 ± 0.18 mmol CO_2 mol^{-1} H_2O
CO_2 compensation point (Γ)	0.5 ± 0.7 µbar
Carboxylation efficiency	0.56 ± 0.17 µmol m^{-2} s^{-1} μbar^{-1}
Enzyme activities[2]	
Rubisco	38.1 ± 0.7 µmol m^{-2} s^{-1}
PEP carboxylase	188.5 ± 10.6 µmol m^{-2} s^{-1}
PEP carboxylase/Rubisco	4.9 ± 0.25 µmol m^{-2} s^{-1}
NADP-malic enzyme	1.9 ± 0.9 µmol m^{-2} s^{-1}
NAD-malic enzyme	43.7 ± 2.3 µmol m^{-2} s^{-1}
PEP carboxykinase	3.0 ± 1.5 µmol m^{-2} s^{-1}

Note: Leaf gas exchange measurements were conducted at 30°C, an ambient CO_2 partial pressure of 394 µbar, and a light intensity of 1800 µmol photons m^{-2} s^{-1}. Standard error is given after mean. *Abbreviations*: PEP, Phosphoenolpyruvate; NADP-malic, Malate dehydrogenase (oxaloacetate-decarboxylating); NAD-malic, Malate dehydrogenase (decarboxylating).

[1] N = 4 for gas exchange.

[2] N = 5 for enzyme assays.

[3] C_i/C_a = the ratio of intercellular to ambient CO_2 partial pressure in µbar.

A/C_i response above a distinct CO_2 saturation point, while C_4-like species such as *Flaveria brownii* (Asteraceae) and lineages with few species such as *Anticharis* (Scrophulariaceae) and *Flaveria kochiana* (Asteraceae) show an upward drift of *A* at elevated CO_2 levels (Khoshravesh et al., 2012).

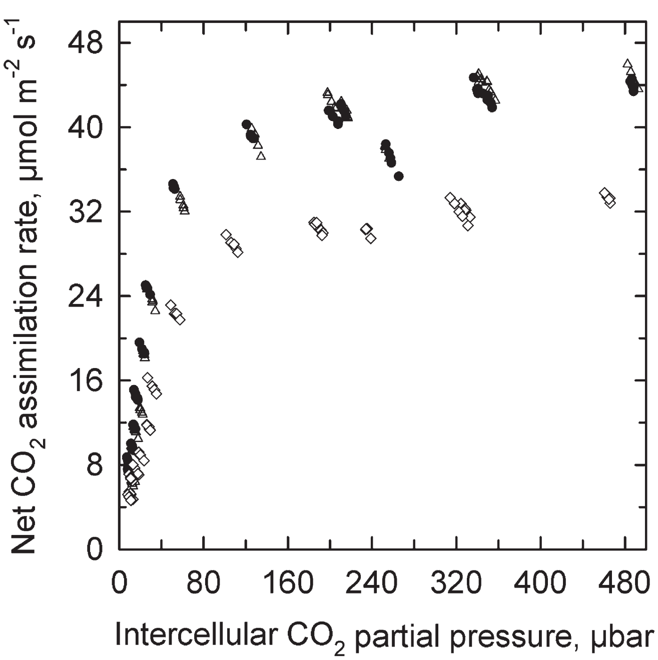

Fig. 5. The response of net CO_2 assimilation rate to the partial pressure of intercellular CO_2 (the A/C_i response) in *Gisekia pharnaceoides* at 30°C and saturating light intensities. Three response curves are represented by different symbols (square, triangle, circle). The CO_2 compensation point (Γ) of four *A/Ci* responses averaged 0.5 ± 1.7.

***Evolution of C_4 photosynthesis in* Gisekia—**The origin of Gisekiaceae dates back to the Early Oligocene/Eocene Epoch (Fig. 2). The fact that all *Gisekia* species perform C_4 photosynthesis suggests that the pathway arose at some point along the stem branch. Therefore, the minimum age for the origin of C_4 photosynthesis would be 14.9 mya (95% confidence interval: 25.7–6.2 mya), meaning that the C_4 pathway arose during the Miocene Epoch in this lineage. This is older than the previous dating of the C_4 origin in *Gisekia* by Christin et al. (2011a), who estimated the maximum age for C_4 in *Gisekia* to be 4.8 mya (± 3.6). This difference might be an effect of the different position of *Gisekia* in the phylogenetic tree of this study in comparison to Christin et al. (2011a). In Christin et al. (2011a: suppl. Fig. 1A) *Gisekia* is sister to *Rivinia* (Phytolaccaceae) while in this study the genus groups clearly outside a clade consisting of Phytolaccaceae, Nyctaginaceae and Aizoaceae (Fig. 2). In *Gisekia*, the physiologically suboptimal C_4 mechanism hypothesized above cannot be explained by a relatively young age of the C_4 lineage.

Southern Africa is one of five distinct geographical centers of C_4 evolution in eudicots (R. Sage et al., 2011). Indigenous C_4 lineages of Southern Africa include C_4 *Cleome* (Cleomaceae) (Feodorova et al., 2010), C_4 *Anticharis* (Scrophulariaceae) (Khoshravesh et al., 2012), C_4 *Mollugo* (Molluginaceae) (Christin et al., 2011b) and C_4 *Zygophyllum* (Zygophyllaceae) (Bellstedt et al., 2012). *Gisekia* may be of South African origin as well (Fig. 2). The tree topology allows two most parsimonious biogeographical hypotheses: (1) A South African origin with subsequent spread to East Africa and another recent spread to South Africa or (2) South African origin with two subsequent migrations/dispersals events to East Africa (Fig. 2). Other C_4 lineages showing a similar biogeographical pattern include *Zygophyllum simplex* (Bellstedt et al., 2012) and C_4 species of *Anticharis* (Khoshravesh et al., 2012). *Gisekia* and *Zygophyllum simplex* are often found growing together in temporary riverbeds or dry valleys (H. Hartmann, personal observations).

The geographical and bioclimatic survey of *Gisekia* reveals a broad climatic niche with ranges of 14–30°C mean annual temperature and 46–794 mm mean annual precipitation, showing that the genus is able to grow in a wide range of extremely arid to mesic habitats (Fig. 1). At the same time, herbarium labels indicate that the genus is closely associated with open and disturbed habitats with low vegetation cover and therefore relatively low competition. Because of its distribution area it might qualify as a member of the arid track (Balinsky, 1962). South Africa and East Africa have been connected through the so-called 'Arid Corridor' intermittently since the Miocene Epoch (Verdcourt, 1969; Jürgens, 1997; Caujapé-Castells et al., 2001; Bellstedt et al., 2012). The stem of the East African *Gisekia* clade dates back to the Miocene/Pliocene epochal boundary (Fig. 2) suggesting that multiple migrations along the 'Arid Corridor' since this time might have been possible. Drier climatic conditions might have facilitated migration for *Gisekia* due to a higher abundance of open habitats. Open habitats, hot temperatures (at least 27°C) and sufficient moisture are required for germination of *Gisekia* seeds (Joshi and Kambhoj, 1959). In contrast to other taxa with a distinctly disjunct distribution pattern (see Jürgens, 1997: Fig. 2) such as *Tribulocarpus* (Thulin, 2012) or *Commicarpus* (Thulin, 1990), *Gisekia* shows a more or less continuous distribution from south to east in Africa and also a spread into arid regions of North Africa and Asia (Fig. 1), indicating that this genus is capable of efficient and rapid dispersal, such as along permanent or temporary streams. The incongruent positions of some accessions in the cp tree (Fig. 2) and in the ITS tree (Appendix S6) suggest that occasional gene flow among the *Gisekia* lineages occurred in the past and therefore might be interpreted as the result of ancient hybridization. Alternatively, incongruent patterns between plastid and nuclear data sets could also be explained by incomplete lineage sorting. The two alternative explanations for incongruencies among gene trees are difficult to discern (e.g., Pelser et al., 2010). The biogeographical history of *Gisekia* supported by the cp data is, however, similar to what has been found in a molecular study of *Androcymbium* Willd. (Colchicaceae) (Caujapé-Castells et al., 2001), a genus of pioneer geophytes distributed in dry areas of southern and northern Africa. *Gisekia* thus gives further support to the conclusion of Caujapé-Castells et al. (2001) that a continuous arid track connecting southern and northern Africa has existed since the late Miocene Epoch.

***Phylogeny and systematics of* Gisekia—**Prior to the revision of Gilbert (1993), two widely distributed species of *Gisekia*, *G. pharnaceoides* with five stamens and *G. africana* with 10-15 stamens, were distinguished. The other five species were described on the basis of differences in inflorescence morphology, pedicel length and mericarp surface structure (Gilbert, 1993). Our phylogenetic analyses covered all known species and included multiple samples for the widespread species. Surprisingly, we found that all species (except *G. paniculata*, which was only sampled once) are polyphyletic in nuclear and cpDNA based molecular trees (Fig. 2, Appendix S6). We failed to find morphological characters that support the clades. Instead we found stamen number (mapped onto the tree in Fig. 2), inflorescence type, and mericarp surface structure to be polymorphic within lineages in *Gisekia*. We also found that distinct morphotypes with, for example, winged mericarps or particularly long pedicels occur in several clades. Also stamen number, which was already used as a diagnostic character prior to Gilbert´s revision, changes frequently within lineages and seems to be evolutionarily labile or even phenotypically plastic (Fig. 2). Considering its stressful habitat and short generation time, rapid changes of the phenotype might be expected in *Gisekia*, but further analyses need to clarify the variability of morphological characters within populations. Incongruencies between plastid and nuclear trees (Fig. 2; Appendix S6) indicate gene flow among lineages of *Gisekia* in the past. The molecular results presented, however, indicate that alternatively to the current recognition of seven species after Gilbert (1993), *Gisekia* is better classified as only containing one polymorphic species, *G. pharnaceoides,* or as a complex with a number of genetically distinct but morphologically cryptic species.

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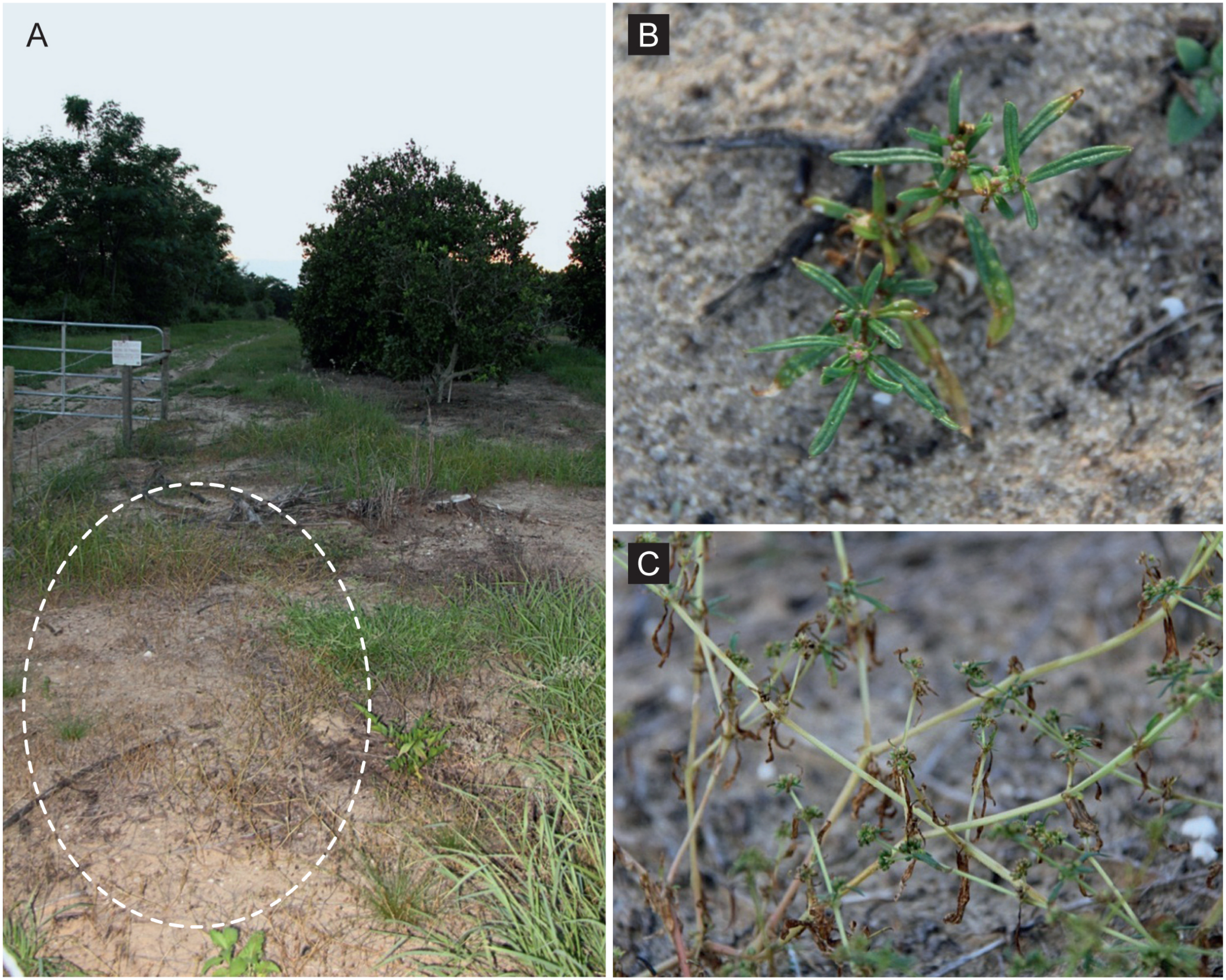


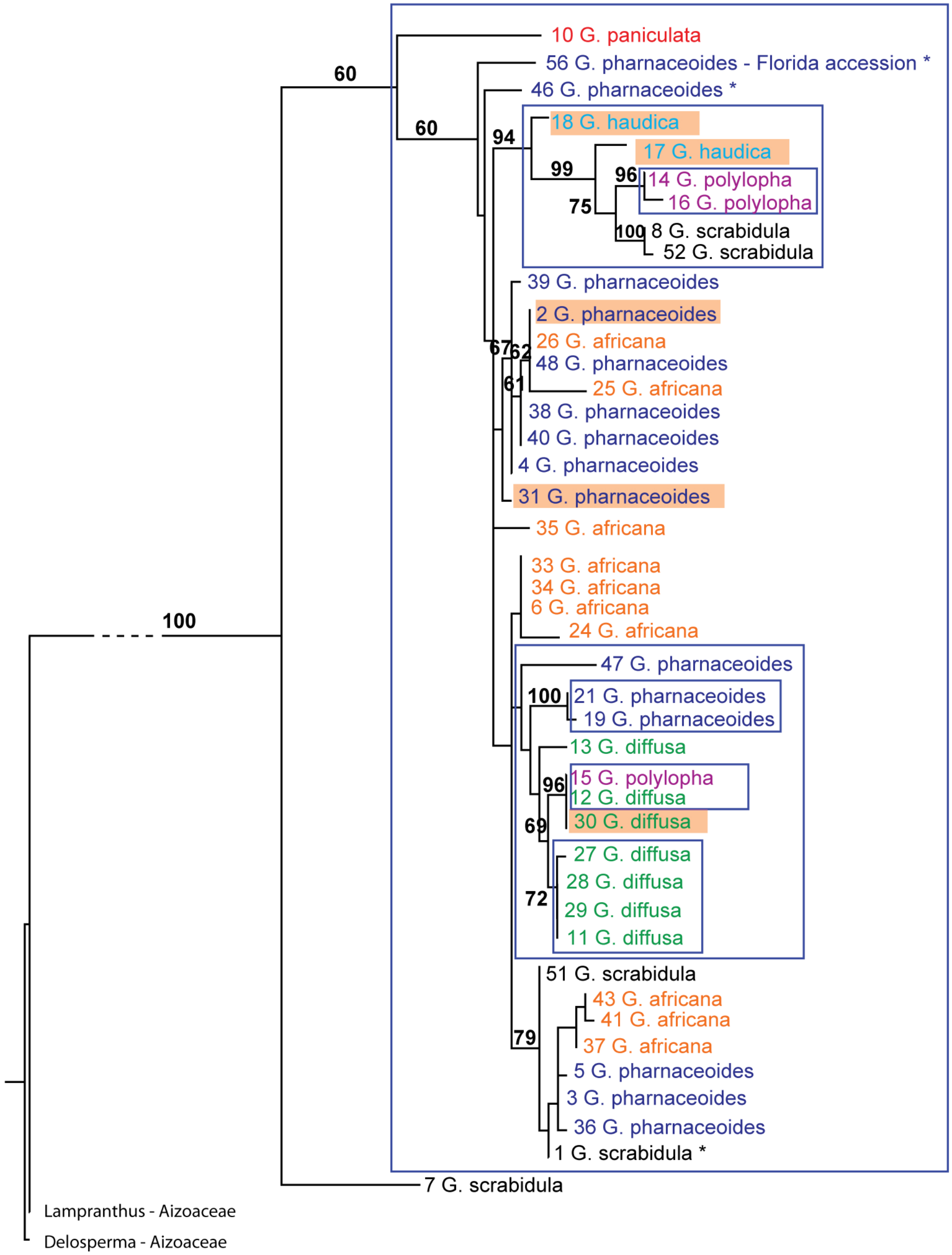
Appendix S1: A) Collection site and habitat of *Gisekia pharnaceoides* on the western outskirts of Orlando, Florida. The dried plants in the white oval are *G. pharnaceoides* plants, the trees are citrus. B) A young and C) older *G. pharnaceoides* plant at the collection site. Note the sandy substrate below the plant in panel B.

Appendix S2: Specimens studied to infer distribution area, bioclimatic preferences, carbon isotope ratio, DNA sequence variation (specimens with a lab code were used for DNA extraction, specimens marked blue were used for anatomical studies and specimens marked orange were used for carbon isotope measurements); **outgroups are added at the end of the table**

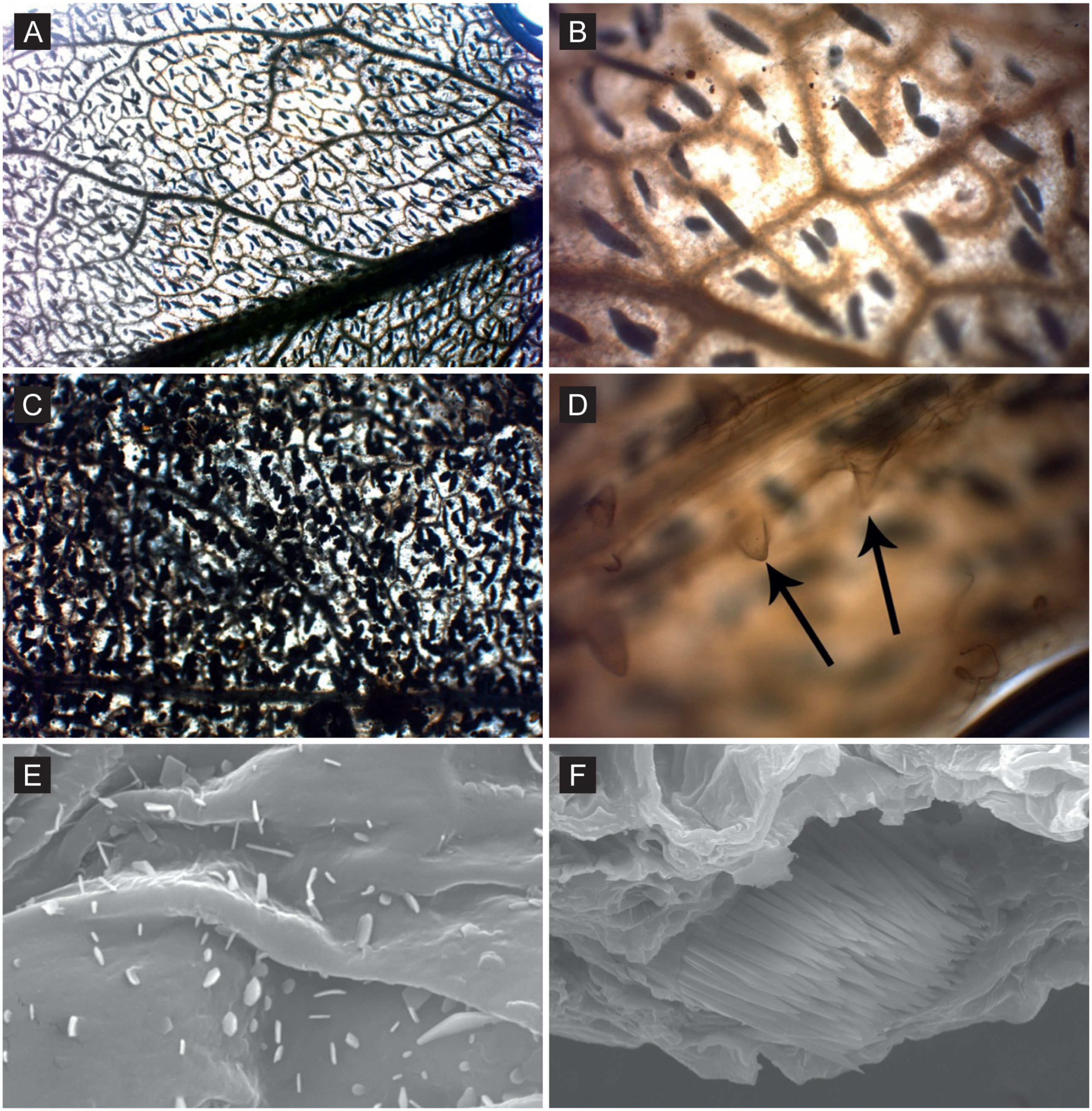
Lab code Gise#	Internal code	Taxon	Genbank accession number					Herbarium Acronym	Collector	Coll. No.	Herbarium No.	Coll. Date	Country	Locality	Latitude (N/S)	Longitude (E/W)	Altitude	dC-value
			atpß-rbcL	matK	petB-petD	rpL16	ITS											
2	H5_p	G. pharnaceoides L.	**KF924286**	**KF995412**		**KF995455**	**KF944398**	HBG	Hartmann	33079		15.02.97	South Africa	Phakane, ca. 12km eastward Kuruman Bophutswana/ North West Province	-27.433333	23.566667	1410 m	
3	H4_p	G. pharnaceoides L.	**KF924293**	**KF995418**	**KF995374**	**KF995463**	**KF944406**	HBG	Hartmann	33169		22.02.97	South Africa	Allemanskraal, Graaff Reinet-Aberdeen Graaf Reinet/Eastern Cape	-32.7	24.433333	620 m	
4	H33_p	G. pharnaceoides L.	**KF924310**	**KF995436**	**KF995392**	**KF995482**	**KF944425**	HBG	Hartmann	32898		07.03.96	South Africa	Wamberg, W of Skeurberg Gordonia/Northern Cape	-28.333333	22.25	1150 m	
5	H34_p	G. pharnaceoides L.	**KF924311**	**KF995437**	**KF995393**	**KF995483**	**KF944426**	HBG	Hartmann	32904		08.03.96	South Africa	Griquastad to Florisfontein, junction to Kalkfontein Hay/ Northern Cape	-28.916667	23.366667	1320 m	
6	H14_aa	G. africana (Lour.) Kuntze var. africana	**KF924312**	**KF995438**	**KF995394**	**KF995484**	**KF944427**	HBG	Hartmann	32873		04.03.96	South Africa	Koegas, between Minenstadt and Ort Hay/Northern Cape	-29.283333	22.383333	980 m	-15,76±0,12
10	H1_d	G. diffusa M. Gilbert	**KF924278**	**KF995403**	**KF995361**	**KF995446**	**KF944389**	HBG	Hartmann	32876		05.03.66	South Africa	Rudesheim, Syferkloof Hay/Northern Cape	-28.966667	22.75	1300 m	
11	U1_d	G. diffusa M. Gilbert		**KF995404**	**KF995362**	**KF995447**	**KF944390**	UPS	Friis, Tadesse & Vollesen	2940	V-539530	15.05.82	Ethiopia	Oromia: Sidamo: Melca Guba, at Darwa Parma River	4.8595	39.323	850m	-12,48±0,03
12	U3_d	G. diffusa M. Gilbert	**KF924279**	**KF995405**		**KF995448**	**KF944391**	UPS	Gilbert, Sebsede & Vollesen	8305	V-539599	19.06.86	Ethiopia	Oromia: Bale: Sof Omar canyon, 2 km away from Web River crossing towards Ginir	6.9	40.83333		
14	U3_y	G. polylopha M. Gilbert	**KF924281**	**KF995407**	**KF995364**	**KF995450**	**KF944393**	UPS	Thulin, Hedrén & Dahir	7318	V-051235/ 43015	08.05.90	Somalia	Galguduud: 7 km along the road from Ceelbur to Ceeldheer	4.633333	46.650000	120 m	
16	U2_y	G. polylopha M. Gilbert	**KF924308**	**KF995433**	**KF995388**	**KF995478**	**KF944421**	UPS	Elmi	108	V-539632	10.11.87	Somalia	Bargaan	4.3666667	47.05	133 m	
17	U2_h	G. haudica M. Gilbert	**KF924283**	**KF995409**	**KF995366**	**KF995452**	**KF944395**	UPS	Thulin & Warfa	4537	V-538453	08.05.83	Somalia	Shabeellaha Hoose: ca. 11km SW. Merka[Marka], near Sanbuusi Hotel	1.66667	44.75	5 m	
18	U1_h	G. haudica M. Gilbert	**KF924284**	**KF995410**	**KF995367**	**KF995453**	**KF944396**	UPS	Thulin & Warfa	5365	V-539534	20.11.85	Somalia	Galguduud: 30 km along the road from Dusab Mareb to Galkayo	5.75	46.53333	250 m	
19	H11_p	G. pharnaceoides L.	**KF924285**	**KF995411**	**KF995368**	**KF995454**	**KF944397**	HBG	Hartmann	21635		27.11.87	Sudan	Jebel Elba, N Hang	22.183333	36.35	400 m	
20	H14_p	G. pharnaceoides L.						HBG	Hartmann	21596		24.11.87	Sudan	8 km N Sufeia=Salala	21.35	36.283333	400 m	
21	H15_p	G. pharnaceoides L.	**KF924287**		**KF995369**	**KF995456**	**KF944399**	HBG	Hartmann	21526		21.11.87	Sudan	Suakin to Tokar, ca.7km S Suakin	19.05	37.3	20 m	
22	H16_p	G. pharnaceoides L.						HBG	Hartmann	21513		20.11.87	Sudan	Sinkat to Gebeit 7km, Khor area	18.9	36.833333	850 m	
23	H3_aa	G. africana (Lour.) Kuntze (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25469		28.02.88	Namibia	Mukorob, Vingerklip Gibeon	-25.5	18.25	1048 m	
24	H15_aa	G. africana (Lour.) Kuntze var. africana	**KF924288**		**KF995370**	**KF995457**	**KF944400**	HBG	Hartmann	32888		06.03.96	South Africa	Rooisand eastward Groblershoop Gordonia/Northern Cape	-28.833333	22.05	900 m	
25	H12_aa	G. pharnaceoides L.	**KF924289**	**KF995413**		**KF995458**	**KF944401**	HBG	Hartmann	31106		14.02.93	South Africa	Sandveld Nature Reserve Hoopstad/Free State	-27.733333	25.65	1120 m	
26	H13_aa	G. africana (Lour.) Kuntze var. africana		**KF995414**	**KF995371**	**KF995459**	**KF944402**	HBG	Hartmann	31267		03.03.93	South Africa	Kuruman, am Dam an Ausfahrt nach Kimberley Kuruman/Northern Cape/Südafrika	-27.466667	23.433333	1000 m	-12,33±0,47
27	U5_d	G. diffusa M. Gilbert	**KF924290**	**KF995415**	**KF995372**	**KF995460**	**KF944403**	UPS	Thulin &Dahir	6413	V-48612/37264	22.05.89	Somalia	Hiraan: 14km along the road between Jalalaqsi and Ceel Baraf	3.316667	45.683333	125-150m	-14,69±1,27
28	U6_d	G. diffusa M. Gilbert	**KF924291**	**KF995416**	**KF995373**	**KF995461**	**KF944404**	UPS	Thulin,Hedrén & Dahir	7319	V-51236/43016	08.05.90	Somalia	Galguduud: 7 km along the road from Ceelbur to Ceeldheer	4.633333	46.65	120 m	-12,75±0,24
29	Ø1_l	G. paniculata Haumann	**KF924292**	**KF995417**		**KF995462**	**KF944405**	C	Gilbert, Sebsede & Vollesen	8305	15	19.06.86	Ethiopia	Bale, Sof Omar Gorge, 2km from Web River junction towards Ghinir	6.9	40.83333	1350 m	-11,65±0,11
30	Ø2_l	G. paniculata Haumann	**KF924294**	**KF995419**	**KF995375**	**KF995464**	**KF944407**	C	Friis,Tadesse & Vollesen	2940	16	15.05.82	Ethiopia	Sidamo, Melca Guba on Dawa Parma River	4.8595	39.323	850 m	
31	H8_p	G. pharnaceoides L.	**KF924295**	**KF995420**	**KF995376**	**KF995465**	**KF944408**	HBG	Hartmann	31308		07.03.93	Botswana	4 km away from Molepolole junction to Letlhakeng Kwenang District	-24.366667	25.4	1150 m	
32	H9_p	G. pharnaceoides L.						HBG	Hartmann	21669		29.11.87	Sudan	43 mls von Dungulab, das 15 km vom Abzweig nach Mohamed Gol//Sudan	21.816667	36.833333	35 m	
33	H4_aa	G. africana (Lour.) Kuntze var. africana	**KF924296**	**KF995421**	**KF995377**	**KF995466**	**KF944409**	HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25465		28.02.88	South Africa	Klein Karas to Keetmanshoop, 10,8 km S from Löwenfluß Keetmanshoop	-27.866667	18.1	860 m	
34	H5_aa	G. africana (Lour.) Kuntze var. africana	**KF924297**	**KF995422**	**KF995378**	**KF995467**	**KF944410**	HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25462		28.02.88	South Africa	Klein Karas to Keetmanshoop, 56,4 km Keetmanshoop	-27.15	18.316667	1210 m	-14,25±0,18

35	H8_aa	G. africana (Lour.) Kuntze var. africana	**KF924298**	**KF995423**	**KF995379**	**KF995468**	**KF944411**	HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25558		06.03.88	South Africa	Luitzputs to Keimoes, 13,2 km from Lutzputs Gordonia/Northern Cape	-28.466667	20.75	850 m	-13,77±0,07
36	H31_p	G. pharnaceoides L.	**KF924299**	**KF995424**	**KF995380**	**KF995469**	**KF944412**	HBG	Hartmann	32868		04.03.96	South Africa	Boesmansberg Prieska/Northern Cape	-29.883333	26.366667	1140 m	
37	U11_aa	G. africana (Lour.) Kuntze var. africana	**KF924300**	**KF995425**	**KF995381**	**KF995470**	**KF944413**	UPS	Skarpe	S-259	V-538986	15.02.78	Botswana	Ghanzi: 9 km W from Ghanzi along the road to Mamuno.	-24.71667	21.56667	1092 m	-16,08±0,82
38	U26_pa*	G. pharnaceoides L. var. alata	**KF924301**	**KF995426**		**KF995471**	**KF944414**	UPS	Skarpe	S-128	V-539606	01.02.77	Botswana	Ghanzi: 4 km N Dondong bore hole.	-23.11667	20.53333	1268m	
39	U27_pa	G. pharnaceoides L. var. alata	**KF924302**	**KF995427**	**KF995382**	**KF995472**	**KF944415**	UPS	Skarpe	S-366	V-539605	30.11.79	Botswana	Ghanzi: 4 km N Dondong bore hole.	-23.11667	20.53333	1268m	
40	U26_pa	G. pharnaceoides L. var. alata	**KF924303**	**KF995428**	**KF995383**	**KF995473**	**KF944416**	UPS	Skarpe	S-364	V-539604	30.11.79	Botswana	Ghanzi: 4 km N Dondong bore hole.	-23.11667	20.53333	1268m	
41	U10_aa	G. africana (Lour.) Kuntze	**KF924304**	**KF995429**	**KF995384**	**KF995474**	**KF944417**	UPS	Skarpe	S-214	V-539528	19.12.77	Botswana	Ghanzi: 53 km NE Ghanzi along the road to Maun.	-22.08333	21.4333	1137 m	-13,49±0,07
42	U27_p	G. pharnaceoides L. var. pharnaceoides						UPS	Skarpe	S-254	V-538995	12.02.78	Botswana	Ghanzi: 5 km NNW Dondong Bohrloch.	-23.11	20.51667	1270 m	
43	ø2_a	G. africana (Lour.) Kuntze	**KF924305**	**KF995430**	**KF995385**	**KF995475**	**KF944418**	C	Reb	7939	2	18.01.1970	South Africa	Botswana District SW Ghanzi Commune Hido Store	-22.127945	21.525249		
44	H18_p	G. pharnaceoides L.						HBG	Hartmann	21480		17.11.87	Sudan	7 km away from trainstation Barameiyn, 11 km towards railway bridge and 1 km from Erkowit	18.518333	36.8	834 m	
45	H20_p	G. pharnaceoides L.						HBG	Hartmann & Newton	28538		18.02.89	Kenya	Ngurunet, camp site slopes on other side of river	4.166667	35.466667	720m	
46	H22_p	G. pharnaceoides L.			**KF995389**	**KF995479**	**KF944422**	HBG	Hartmann & Newton	28579		18.02.89	Kenya	2km from junction to Barsalinga, 8.2 km from junction to Lodungokwa, 25.2 km from junction to Wamba	0.883333	37.1	1190 m	
47	H23_p	G. pharnaceoides L.	**KF924309**	**KF995434**	**KF995390**	**KF995480**	**KF944423**	HBG	Hartmann & Newton	28515		23.02.89	Kenya	6.5 km E Ilhaut	1.883333	37.216667	730 m	
48	H28_p	G. pharnaceoides L.		**KF995435**	**KF995391**	**KF995481**	**KF944424**	HBG	Hartmann	31302		07.03.93	Botswana	ca. 5 km E Letlhakeng Kwenang District	-24.133333	25.133333	1150 m	
1&51	H1_y +H1_s	G. polylopha M. Gilbert und scrabidula M. Gilbert	**KF924277**	**KF995402**	**KF995360**	**KF995445**	**KF944388**	HBG	Hartmann	34747		04.03.06	South Africa	Biesjiesfontein nearHutchinson Victoria West/Northern Cape	-31.5	23.183333		
13 & 50	U4_d	G. diffusa M. Gilbert	**KF924280**	**KF995406**	**KF995363**	**KF995449**	**KF944392**	UPS	Gilbert & Sebsede	8696	V-539533	05.06.88	Ethiopia	Oromia: Sidamo: 2 km from Dawa River and Melca Guba along the road to Negele Borena	4.86667	39.36667	850m	
15 & 49	U1_y	G. africana (Lour.) Kuntze	**KF924282**	**KF995408**	**KF995365**	**KF995451**	**KF944394**	UPS	Friis, Gilbert & Vollesen	3666	V-538459	31.10.84	Ethiopia	Oromia: Bale A. Region: near Sof Omar Caves	6.91667	40.83333	1400 m	
7 & 53	H3_s	G. scrabidula M. Gilbert	**KF924307**	**KF995432**	**KF995387**	**KF995477**	**KF944420**	HBG	Hartmann	25118		09.02.88	South Africa	Mkuze Park, Ubombo/KwaZulu/Natal	-27.65	32.183333	100 m	
8 & 52	U2_s	G. scrabidula M. Gilbert	**KF924306**	**KF995431**	**KF995386**	**KF995476**	**KF944419**	UPS	Thulin & Dahir	6432	V-048631/ 37283	22.05.89	Somalia	Hiraan: 6kmalong the road between Ceel Baraf and Adan Yabal	3.25	45.8	150 m	
9&57	U1_s	G. scrabidula M. Gilbert						UPS	Kasmi, Marion & Istambal	5364	V-539630	25.06.83	Somalia	Hiraan: Mahaas towards Bulloburte (Buloburde)	3.85	45.575833		
	H19_p	G. pharnaceoides L.						HBG	Hartmann	32877		05.03.66	South Africa	Rudesheim, Syferkloof Hay/Northern Cape	-28.966667	22.75	1300 m	
	H17_p	G. pharnaceoides L.						HBG	Hartmann	21495		18.11.87	Sudan	old road from Sinkat to Erkowit, 3 miles from junction	18.766667	36.85		
	H12_p	G. pharnaceoides L.						HBG	Hartmann	21600		24.11.87	Sudan	Khor 7miles N Sufeia=Salala	21.333333	36.25	450 m	
	H13_p	G. pharnaceoides L.						HBG	Hartmann	21601		##.##.####	Sudan	Khor 7miles N Sufeia=Salala	21.333333	36.25		
	H10_p	G. pharnaceoides L.						HBG	Hartmann	21626		26.11.87	Sudan	27 km towards Halaib	21.9	36.833333	400 m	
	H6_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann	25122		09.02.88	South Africa	Stanmore, Dubula Wildplaas SW Golela Ngotshe/KwaZulu/Natal	-27.366667	31.833333	200 m	-14,36±0,44
	H2_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25477		01.03.88	Namibia	S Windhoek:Kaminskiberg E Auas Born Windhoek	-22.666667	17.133333	2178 m	-12,66±0,05
	H1_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25482		01.03.88	Namibia	4.8 km N Kalkrand Rehoboth	-24.05	17.566667	1200 m	-13,26±0,05
	H7_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25486		01.03.88	Namibia	Gochas to Keetmanshoop Gibeon	-25.366667	18.65	1100 m	-13,24±0,35
	H7_aa*	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25551		06.03.88	South Africa	Ariamsvlei to Upington 10,4 km from junction to Lutzputs Gordonia/Northern Cape	-28.283333	20.566667		-13,65±0,07
	H10_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25571		06.03.88	South Africa	Louisvale, c.8 km S Abzweig nach Kenhardt Gordonia/Northern Cape	-28.6	21.233333	790 m	
	H9_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25606		07.03.88	South Africa	Maraisvlei Kenhardt/Northern Cape	-28.866667	20.316667	950 m	-14,16±0,08
	H11_aa	G. africana (Lour.) Kuntze var. africana						HBG	Hartmann, Dehn, Gölling, Rust & Stüber	25646		08.03.88	South Africa	Pofadder nach Aggeneys, Spioenkop Namaqualand/Northern Cape	-29.183333	19.05	880 m	-12,87±0,04

	H21_p	G. pharnaceoides L.						HBG	Hartmann & Newton	28548		18.02.89	Kenya	Kurungu Camp Site nach Kargi,2,7 km from junction; 11,6km from Camp Site	2.283333	36.9		
	H39_p	G. pharnaceoides L.						HBG	Hartmann	31217		25.02.93	South Africa	Danielskuil, Camp 17 Postmasburg/Northern Cape	-28.15	23.583333	1550 m	
	H24_p	G. pharnaceoides L.						HBG	Hartmann	31224		25.02.93	South Africa	Danielskuil, road below Reservois Postmasburg/Northern Cape	-28.166667	23.55	1500 m	
	H29_p	G. pharnaceoides L.						HBG	Hartmann	31270		03.03.93	South Africa	Kuruma to Vryburg, ca. 90 km towards Vryburg Bophutsatswana/North West Province/	-27.3	23.916667	1460 m	
	H25_p	G. pharnaceoides L.						HBG	Hartmann	31273		03.03.93	South Africa	Zoetvlei Vryburg/North West Province/Südafrika	-27.1	24.233333	1400 m	
	H27_p	G. pharnaceoides L.						HBG	Hartmann	31288		06.03.93	Botswana	E Phitsame Molopo,Matletse Ngwaketse District	-25.716667	25.05	1150 m	
	H2_s	G. scrabidula						HBG	Hartmann	31305		07.03.93	Botswana	ca. 5 km E Letlhakeng Kwenang District/Botwana	-24.133333	25.133333	1150 m	
	H7_p	G. pharnaceoides L.						HBG	Hartmann	31310		07.03.93	Botswana	Kolokeng Mission Kwenang District	-24.666667	25.616667	1150 m	
	H30_p	G. pharnaceoides L.						HBG	Hartmann	32792		26.02.96	South Africa	Oudefontein Albert/Eastern Cape/Südafrika	-30.55	28.3255	1300 m	
	H32_p	G. pharnaceoides L.						HBG	Hartmann	32897		07.03.96	South Africa	Roipan Gordonia/Northern Cape	-28.433333	22.233333	1050 m	
	H35_p	G. pharnaceoides L.						HBG	Hartmann	33007		06.02.97	South Africa	Wonderfontein Road, Abzweig nach Mullersrus Sasolburg/Free State	-26.783333	27.766667	1450 m	
	H26_p	G. pharnaceoides L.						HBG	Hartmann	33046		11.02.97	South Africa	Rodemanskloof,Ostzaun Hay/NorthernCape	-28.633333	22.666667	1000 m	
	H36_p	G. pharnaceoides L.						HBG	Hartmann	33060		13.02.97	South Africa	Kalkfontein, am Haus Hay/Northern Cape/Südafrika	-28.933333	23.316667	1350 m	
	H38_p	G. pharnaceoides L.						HBG	Hartmann	33068		14.02.97	South Africa	Postmasburg, Hospital Hill Postmasburg/Northern Cape	-28.333333	23.05	1300 m	
	H6_p	G. pharnaceoides L.						HBG	Hartmann	33068		##.##.####	South Africa	Postmasburg Hospital Hill Postmasburg	-28.333333	23.05		
	H3_p	G. pharnaceoides L.						HBG	Hartmann & Mills	34754		10.02.06	South Africa	Ditholo Nature Reserve Hammanskraal/ Gauteng	-28.323167	28.3255	1070 m	
	H1_p	G. pharnaceoides L.						HBG	Hartmann	21577		##.##.####	Sudan	Khor Region	21.033333	36.35	570 m	
	H2_p	G. pharnaceoides L.						HBG	Hartmann	25552		##.##.####	South Africa	Ariamsvlei to Upington 10,4 km from junction to Lutzputs Gordonia	-28.283333	20.566667		
	U1_p	G. pharnaceoides L. var. pharnaceoides						UPS	Kotschy	185	V-539621	##.##.####	Sudan	Shamal Kurdufan	15.186333	29.559167		
	U1_aa	G. africana (Lour.) Kuntze var. africana						UPS	Nels	80	V-539561	01.03.05	Namibia	Hereroland (Tsumkwe)	-19.589167	20.513333		
	U2_aa	G. africana (Lour.) Kuntze var. africana						UPS	Schlechter	4575	V-539577	##.##.####	South Africa	Transvaal: pr. Madsaba.	-25.752845	28.187370		
	U2_p	G. pharnaceoides L.						UPS	Mokim	563	V-539626	##.11.1902	Myanmar	Magway:District Minbu Shingage	20.138167	94.935		
	U3_p	G. pharnaceoides L. var. pharnaceoides						UPS	Fries	86	V-539623	##.07.1911	Sambia	Southern: Rhodesia bor.-occid: Victoria waterfalls	-17.806833	25.786667		
	U4_p	G. pharnaceoides L. var. pharnaceoides						UPS	Fries	960	V-539627	09.10.11	Sambia	Northern Rhodesia bor. Orient.Bangweolo, Kamindas	-10.991333	30.1225		
	U5_p	G. pharnaceoides L. var. pharnaceoides						UPS	Fries	1438	V-539628	10.12.11	Burundi	Ruzizi River between Mpanda and Mercherenge	-3.202833	29.235167		
	U6_p	G. pharnaceoides L. var. pharnaceoides						UPS	Mhoro	284	V-539603	28.01.17	Tanzania	Iringa: Kidatu.	-7.11667	36.15	770 m	
	U7_p	G. pharnaceoides L.						UPS	Borle	291	V-539592	01.02.20	Mosambique	Maputo City: Lourenço Marques (Maputo)	-25.9715	32.5685		
	U3_ad	G. africana (Lour.) Kuntze var. decagyna						UPS	Borle	11512	V-539591	##.08.1920	Mosambique	Maputo City: Lourenço Marques (Maputo)	-25.9715	32.5685		
	U4_aa	G. africana (Lour.) Kuntze var. africana						UPS	Örtendahl		V-539569	##.##.1925	Namibia	Karas: klein Karas	-27.564815	18.076772		
	U5_aa	G. africana (Lour.) Kuntze var. africana						UPS	Örtendahl		V-539576	##.##.1925	Namibia	Karas: klein Karas	-27.564815	18.076772		
	U6_aa	G. africana (Lour.) Kuntze var. africana						UPS	Örtendahl	73	V-539564	11.04.31	Namibia	Karas: Great Namaqualand: Klein Karas	-27.564815	18.076772		
	U7_aa	G. africana (Lour.) Kuntze var. africana						UPS	Örtendahl	92	V-539535	12.04.31	Namibia	Karas: Great Namaqualand: Klein Karas	-27.564864	18.076706	800 m	
	U8_aa	G. africana (Lour.) Kuntze africana						UPS	Örtendahl	91	V-539581	12.04.31	Namibia	Karas: Great Namaqualand: Klein Karas	-27.564762	18.076909	800 m	
	U9_aa	G. africana (Lour.) Kuntze var. africana						UPS	Örtendahl	319	V-539602	30.05.31	Namibia	Great Namaqualand: Ariamsvlei, Wakerobrunn farm	-28.113350	19.827360		
	U8_p	G. pharnaceoides L. var. pharnaceoides						UPS	Kotschy		V-539629	18.08.39	Sudan	Near Abu-Gerad	14.558833	24.918333		
	U9_p	G. pharnaceoides L. var. pharnaceoides						UPS	Faulkner	3622	V-539611	18.08.56	Tanzania	Tanga: Sawa	-5.003333	38.907667		
	U10_p	G. pharnaceoides L. var. pharnaceoides						UPS	Moggi & Bavazzano	116	V-539595	22.07.59	Somalia	between Mogadishu and Afgoi	2.089	45.203333		



Appendix S6: Maximum likelihood phylogram of *Gisekia* based on the nuclear ribosomal marker ITS, numbers above branches represent ML bootstrap values (>60), numbers in terminals represent lab codes. The tree was rooted with two representatives of Aizoaceae (*Lampranthus bicolor* and *Delospema echinatum*). Terminals marked with * are not represented in the tree based on cp DNA data (Fig. 2). Terminals with orange boxes represent accessionswith supported conflicting positions in the cp tree. Blue boxes represent clades also supported in the cp tree.



Appendix S7: A) and B) The lower leaf surface of *G. pharnaceoides* (UPS: V539615) showing the density of secondary and tertiary veins and the distribution of raphide idioblasts between them. C) Lower leaf surface of *G. diffusa* (UPS: V538459) showing a much higher density of raphide idioblasts. D) Lower leaf surface of *G. scabridula* (UPS: V048631) with tooth-shaped protrusions on the mid-vein. These are also present on a specimen of *G. pharnaceoides* subsp. *alata* (UPS: V-539605). E) Wax platelets on the cuticle of *G. pharnaceoides* (UPS: V539605). F) Idioblast with bundle of raphides (*G. pharnaceoides* (UPS: V539605))

Appendix S5. δ^{13}C values of *Gisekia.*

Species	Appendix code and Lab code[1]	δ^{13}C values	Voucher (collector + no, country, herbarium)
G. africana	#	-12.7	Curtis 59, Mozambique, GH
G. africana	#	-14.3	Strid 2407, Zambia, MO
G. africana	#	-12.2	Takaindisa 8, Rhodesia, AA
G. africana	#	-13.1	Taylor et al 8499, Tanzania, MO
G. africana	H5aa, Gise34	-14,3± 0,2	Hartmann et al. 25462, South Africa, Keetmnshoop; HBG
G. africana	H14aa, Gise6	-15,8 ± 0,1	Hartmann 32873, South Africa, Koegas; HBG
G. africana	H11aa	-12,9	Hartmann et al. 25646, South Africa, Namaqualand; HBG
G. africana	H13aa, Gise26	-12,3 ± 0,5	Hartmann 31367, South Africa, Kuruman; HBG
G. africana	H8aa, Gise35	-13,8 ± 0,1	Hartmann et al. 25558, South Africa, Luitzputz Gordonia; HBG
G. africana	H7aa, Gise55	-13,2 ± 0,4	Hartmann et al. 25486, Namibia; HBG
G. africana	H6aa	-14,4 ± 0,4	Hartmann 25122, South Africa, Kwa Zulu Natal; HBG
G. africana	H7aa*	-13,7 ± 0,1	Hartmann et al. 25551, South Africa, Ariamsvlei/Upington; HBG
G. africana	H1aa	-13,3 ± 0,1	Hartmann et al. 25482, Namibia; HBG
G. africana	H2aa	-12,7 ± 0,1	Hartmann et al. 25477, Namibia; HBG
G. africana	H9aa	-14,2 ± 0,1	Hartmann et al. 25606, South Africa, Maraisvlei Kenhardt; HBG
G. africana	Ø1a	-14,3	Strid 2407, Zambia, Barotseland; C
G. africana	Ø6a	-13,8 ± 0,1	Hansen 3337, Botswana; C
G. africana	Ø5a	-12,4 ± 0,2	Elmi & Hansen 4073, Somalia; C
G. africana	Ø4a	-13,4	Bamps et al. 4565, Angola, Tambor; C
G. africana	U15aa	-13,5 ± 0,1	Philipson 846, Kuiseb Pass; UPS
G. africana	U11aa, Gise37	-16,1 ± 0,8	Skarpe S-259, Ghanzi; UPS
G. africana	U10aa, Gise41	-13,5 ± 0,1	Skarpe S-214, Ghanzi; UPS
G. africana	#	-9,2	Örtendahl 319, South Africa; MO
G. africana	U7aa	-13,9	Örtendahl 92, South Africa, Namaqualand; UPS
G. africana	U1y	-13,4	Friis et al. 3666, Oromia; UPS
G. africana var. *decagyna*	U3ad	-12.0	Borle 11512, Mocambique,Maputo; UPS
G. diffusa	U1d, Gise30	-12,5	Friis et al. 2949, Ethiopia, Oromia; UPS.
G. diffusa	U5d, Gise27	-14,7 ± 1,3	Thulin & Dahir 6413, Somalia, Hiraan; UPS
G. diffusa	U6d, Gise28	-12,8 ± 0,2	Thulin et al. 7319, Somalia, Galguduud; UPS
G. haudica	U1h	-12,1	Thulin & Warfa 5365, Galguduud; UPS
G. pharnaceoides	#	-11,6 ± 0,1	Gilbert et al. 8305, Ethiopia; C
G. pharnaceoides	#	-12.4	Marques 11631, Mozambique; MO
G. pharnaceoides	#	-11.4	Macquire 2153, South Africa; H
G. pharnaceoides	#	-12.6	Rodin 4227, South Africa; MO
G. pharnaceoides	#	-11.9	Swans 4263, South Africa; MO
G. pharnaceoides	#	-11.8	Werdemann & Overdieck 1781, South Africa; AA
G. pharnaceoides	#	-10.6	Macquire 2150, South Africa; H
G. pharnaceoides	#	-11.9	Godfrey 57373, Florida, USA; GH

Species	Appendix code and Lab code[1]	$\delta^{13}C$ values	Voucher (collector + no, country, herbarium)
G. pharnaceoides	#	-13.0	Judd 2474, Florida, USA; GH
G. pharnaceoides	#	-13.0	Larsen & Larsen, 33727, N. Thailand; MO
G. pharnaceoides	#	-14.3	MO 3491261, South Africa; MO
G. pharnaceoides	#	-13.2	Rae 45, Botswana; MO
G. pharnaceoides	#	-11.8	Rodin 9036, South Africa; H
G. pharnaceoides	U6p	-13,4	Mhoro 284, Iringa, Kidatu; UPS
G. polylopha	U3y	-14,6	Thulin et al. 7318, Galguduud; UPS
G. scabridula	U2s	-13,8	Thulin & Dahir 6432, Hiraan; UPS

[1]All samples with # for lab code were analyzed at the University of California, Davis isotope facility, the others at the Institut für Geowissenschaften, University Mainz, Germany