Bienertia sinuspersici (Chenopodiaceae): A New Species from Southwest Asia and Discovery of a Third Terrestrial C$_4$ Plant Without Kranz Anatomy

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Abstract. Our studies on the enigmatic genus *Bienertia* (Chenopodiaceae), with its C$_4$ photosynthesis and lack of Kranz anatomy, led us to the discovery of a second species of this previously-supposed monotypic genus. The new species is named ***Bienertia sinuspersici*** after its main range around the Persian Gulf countries and the northern side of the Gulf of Oman. *Bienertia sinuspersici* occurs in hot climates and is a vicariant of *Bienertia cycloptera*, which is found at higher latitudes and elevations in temperate and cold deserts of the region. Like *Bienertia cycloptera*, the new species has unique chlorenchyma cells with dimorphic chloroplasts and single cell C$_4$ photosynthesis. However, it differs anatomically by having mostly one to two layers of chlorenchyma cells, versus two to three layers in *Bienertia cycloptera*. Furthermore, the new species has longer cotyledon leaves, larger seeds, larger flowers, and larger chromosomes, and differs in a set of micro-morphological features. All of this supports our conclusion that this widely distributed, novel plant is an overlooked new species. *Bienertia sinuspersici* grows well in very hot climates, under conditions which most species can barely tolerate. Its wide distribution indicates that its novel C$_4$ photosynthesis may confer advantages for CO$_2$ fixation in these habitats not found in C$_4$ species having conventional Kranz anatomy.

Recently, two species in family Chenopodiaceae, *Bienertia cycloptera* and *Borszczowia aralocaspica*, were found to have a unique mechanism of C$_4$ photosynthesis, which occurs within a single photosynthetic cell (Freitag and Stichler 2000, 2002; Voznesenskaya et al. 2001, 2002). This stimulated us to conduct a broader taxonomic, ecological, and physiological search among plant populations of *Bienertia*, a supposedly monotypic genus (Akhani et al. 2003), in order to further elucidate the ecological importance and limitations of this unique photosynthetic system. The first stage of analysis was concentrated on a population collected by H. Akhani in central Iran (Freitag and Stichler 2002; Voznesenskaya et al. 2002). Physiological and cellular studies demonstrated that *Bienertia* was able to carry out C$_4$ photosynthesis in a single chlorenchyma cell through partitioning of organelles and enzymes into two distinct compartments within the cell, thus achieving the spatial separation of reactions found in the dual cell system of Kranz anatomy (Voznesenskaya et al. 2002). During 2002 and 2003, we extended our studies to populations that originated from southern Iran. From intensive field studies in many localities in Iran, obvious and consistent differences in morphological

and anatomical features were observed between plants in southern Iran and the central Iranian population of *Bienertia cycloptera*. Differences described in this paper indicate the existence of two distinct species of *Bienertia*, and they have led to the discovery of a third terrestrial C$_4$ plant without Kranz anatomy. The newly identified species will be called *Bienertia sinuspersici*, based on its range, which is distinct from that of *B. cycloptera*.

Materials and Methods

Field and Herbarium Studies. The field studies were carried out in different parts of Iran, primarily in central, eastern, and southern Iran. Plants of several populations were collected and archived using conventional herbarium methods. All vouchers have been preserved in the Faculty of Science, Laboratory of Plant Geography and Plant Systematics, University of Tehran, with some duplication in other herbaria. A usual problem in many fleshy Chenopodiaceae is that many important taxonomic characters disappear in dried herbarium specimens. Thus, all parts of the plants were photographed in their natural habitat for further analyses. Furthermore, fresh plants were immediately transferred to the laboratory for further detailed measurements.

Cultivation. In order to determine if the observed morphological and anatomical differences were due to habitat differences, seeds of the species from southern Iran were cultivated in the natural habitat of *B. cycloptera* in three sites located in salt flats

near Mardabad (ca. 40 kilometers west of Tehran). In the first few weeks, they were watered to facilitate higher rates of germination. They were monitored at 2–3 week intervals while measuring their height, and a number of living plants were photographed.

For further anatomical and physiological examination, plants were grown in environmentally controlled chambers (School of Biological Sciences, Washington State University, Pullman, WA, USA). Seeds were first germinated at room temperature in Petri dishes. They were then transplanted to 1 L pots, containing a mixture of 10 parts commercial potting soil, one part clay, one part sand, and 100 grams gypsum, and grown for 3 days under 30 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 30/25℃ day/night temperature regime. Beginning on day 4, plants were grown in a chamber (model GC-16; Enconair Ecological Chambers Inc., Winnipeg, Canada) under a PPFD of approximately 400 μmol m^{-2} s^{-1} with a 16 h/8 h light/dark photoperiod and 25℃/15℃ day/night temperature regime.

Microscopy. For light microscopy, the study of leaf structure was made both on material collected in natural environments and on material grown under controlled conditions in chambers. The plants, newly collected from nature, were studied by stereomicroscopy (Olympus SZ 21) and transmitted light microscopy (Olympus BX51). The leaves were sectioned by hand and studied without staining (in our experience most major features are easily distinguishable under non-stained conditions). The photographs were taken with a digital camera (PE12). Measurements were made with OLYSIA, 2002 software.

Stomatal density (SD, number mm^{-2} leaf area) was determined on epidermis removed from fresh leaves of plants grown in growth chambers. The epidermis of *B. cycloptera* and *B. sinuspersici* was easily detached from leaves and mounted directly in 50% glycerol-water drops on microscope slides and then covered with a cover slip. Stomatal density was measured along the central lateral zone of both abaxial and adaxial sides of two leaves per species, using a Leitz Aristoplan microscope (Leitz, Oberkochen, Germany) coupled to a Sony video system (Sony, Tokyo, Japan). Ten stomatal counts were made per side at a magnification of 10x, within an area of 0.48 mm^2.

Samples for high resolution light microscopy were fixed for 10–24 hr at 4℃ in 2% (v/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The samples were dehydrated with a graded ethanol series and embedded in London Resin White (LR White, Electron Microscopy Sciences, Fort Washington, PA, USA) acrylic resin. Semi-thin cross sections (about 0.8–1 μm thick) were made on a Reichert Ultracut R ultramicrotome (Reichert-Jung GmbH, Heidelberg, Germany). Sections were stained with 1% (w/v) Toluidine blue O in 1% (w/v) Na$_2$B$_4$O$_7$. Light microscopy images were taken using a color Sony CCD camera interfaced to a Leitz Aristoplan photomicroscope.

Pollen morphology was studied according to the acetolysis method (Erdtman 1960) using a Olympus BX51 microscope and the software OLYSIA, 2002. This provides a more precise analysis than in the previous report on *B. cycloptera* (Akhani et al. 2003), in which a normal Zeiss microscope was used with measurements by an objective micrometer.

The micromorphological features of seeds and pollen were studied by scanning electron microscopy (SEM) after gold coating of samples, using a Zeiss DSM 960 SEM (courtesy of Mr. S. M. H. Hashemi). Leaf samples for SEM were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), postfixed in 2% (w/v) OsO$_4$, and then dehydrated in an ethanol series to 100% ethanol, cryofractured in liquid nitrogen then critical-point dried, attached to SEM mounts, sputter-coated with gold and observed with a Hitachi S570 SEM.

Chromosome Studies. The chromosome morphology of the two species was studied by the squash method. Young, germinated seedlings were prefixed in 20 mM 8-hydroxyquinoline for 3 hr at room temperature and then fixed in 6:3:2 ethyl alcohol (96%): chloroform: propionic acid at room temperature for 24 hr. After rinsing with distilled water, root tips were hydrolyzed in 1 N hydrochloric acid for 10 min at 60℃. Staining was carried out with the Feulgen reaction enhanced by squashing in 2% (w/v) acetocarmine. The

Levan et al. (1964) procedure was followed for characterizing chromosome types.

Chlorophyll and Protein Assay. Chlorophyll was extracted in 80% acetone, and analyzed as described in Porra et al. (1989). Absorbance was measured at 646.6 and 663.6 nanometers (nm) on a Perkin-Elmer 552A spectrophotometer with three replicates per sample. For protein assay, leaf material was ground in a Ten-Broeck homogenizer with 1 ml of 50 mM HEPES buffer (N-[2-Hydroxyethyl]piperazine-N′-[ethanesulfonic acid]) (pH 7.5) and 1% polyvinylpyrrolidone. Assays were made by measuring absorbance at 595 nm by the method of Bradford (1976) with bovine serum albumin as a standard.

Carbon Isotope Composition. The carbon isotope composition for both *Bienertia* species was measured at different time intervals, from different organs and from various sites, according to the method in Osmond et al. (1975).

SYSTEMATICS

Bienertia sinuspersici Akhani sp. nov. (Table 1, Fig. 1, in comparison with *B. cycloptera* Bunge ex Boiss.).—TYPE: IRAN. Khuzestan: Between Ahvaz and Abadan, ca. 52 km S of Ahvaz, 6 km N of Darkhoin, 30°46′56″ N, 48°28′1″ E, elevation 20 m, 31 Oct 2003, *Akhani* 17439 (holotypus: IRAN, isotypus: TUH, Hb. Akh.).

Differt ab *Bienertia cycloptera* Bunge ex Boiss. foliis planis (nec cyclindricis), latiore (nec teretia), 5.5–10 (nec 2–4) mm lata, folia cotyledonae ca. duplo longiore, 9–12 (nec 5–6.5) mm longa. Seminibus et tepalis proportione majoribus.

Annual plant, erect, to ascending, stem at the base up to 4 cm diam, very branched beginning from the base, divaricately branched throughout, main stems up to 130 cm tall, in favorable conditions produces dense canopies up to 160 cm diam, light green or becoming reddish by age, normally glabrous, but in young vegetative stage with bladder-like globose to ovoid and shortly stipitate colorless hairs, 0.1–0.15 mm long. Leaves alternate, fleshy, oblong, glaucous, (17-) 20–35 (-41) mm long (mean 27.0 mm long), 5.5–10 mm wide (mean 8.15 mm), midrib prominent, thickness 1.7–2.2 mm (midrib), 1–1.2 mm (lamina), without petiole, flat, surface with a powder-like bloom, in juvenile stage with bladder-like globose to cylindrical hairs, these shed before flowering and fruiting, apex obtuse, upper surface bulging over the midrib. Floral leaves usually shorter than lower leaves. Inflorescence paniculate, flowers on a spike-like shoot arising from the axil of a floral leaf (bract); spikes simple, very rarely branched, 30–50 cm long, with 13–25 flowers per spike, flowers appearing sessile on a very short elevated base. Flowers usually hermaphrodite, rarely unisexual. Perianth at flowering time (prior to development of wings) 2.5–4 mm diam, perianth segments 1.3–1.7 mm wide. Fruiting perianth 7.2–7.5 mm diam, wings 1–1.7 mm; perianth segments connate to 1/2 to 3/4 of their length, obtuse at apex, upper margin with a membranaceous margin ca. 0.25 mm. Stigmas 2–3, clavate, base tapering into an inconspicuous style, styles (including

TABLE 1. Comparison of characters between *Bienertia cycloptera* and *Bienertia sinuspersici*. The standard errors shown after plus-minus symbol "±" are based on the replicates given in parenthesis. * distance between the centers of two adjacent pores.
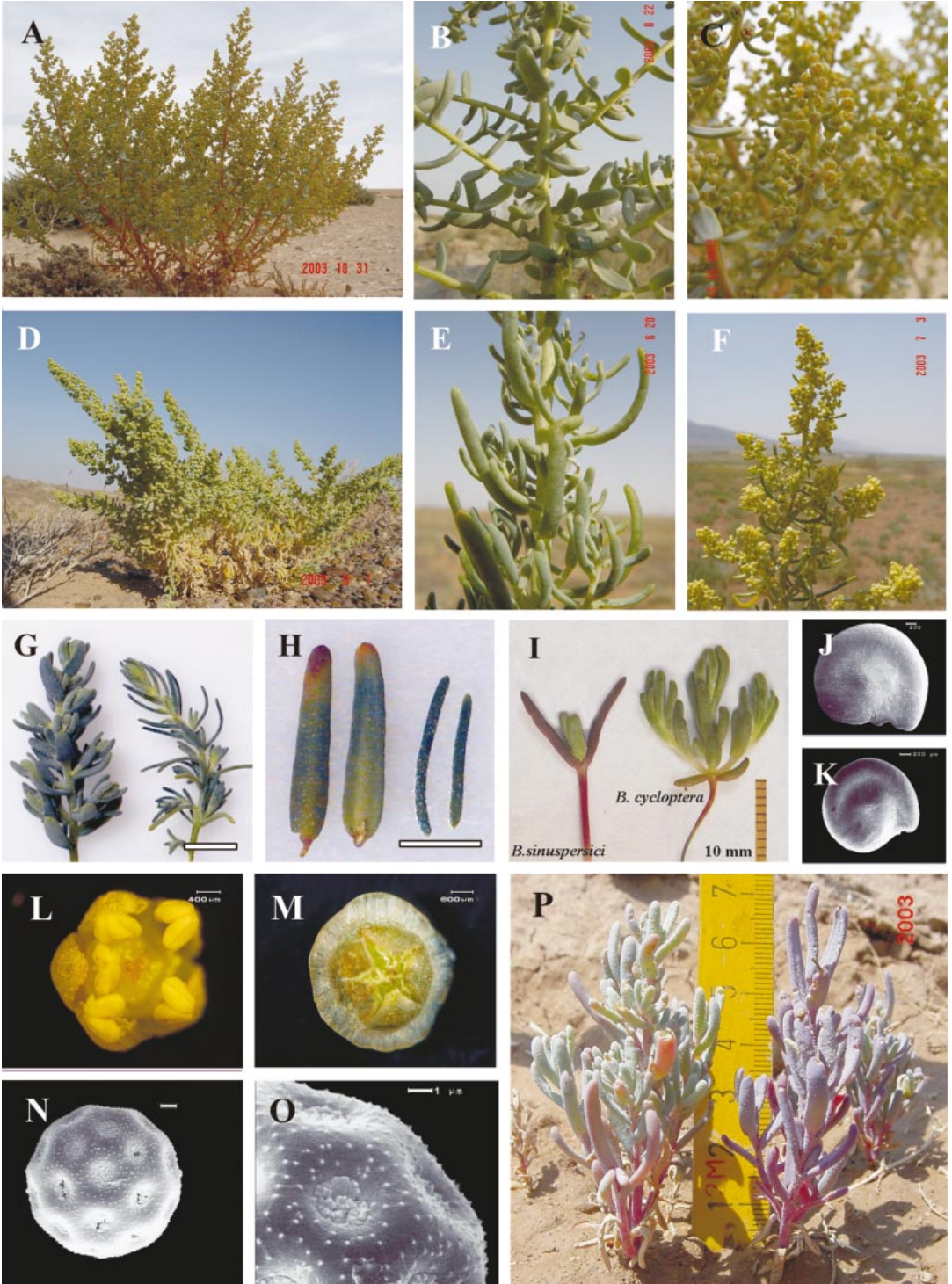
Character	B. cycloptera	B. sinuspersici
	Natural environments	
Distribution	Widespread in Central Iran and Turkmenistan with sporadic occurrence in Turkey and Central Asia	Restricted around Persian Gulf areas and Baluchestan
Plant size (height/diameter of bush) (cm)	Usually shorter, precise measurements from several populations not available	up to 130/160
Flowering time	July–August	October
Fruiting time	September	November–December
Perianth diameter (mm)	4–6.5	7.2–7.5
Pollen diameter (D) (µm)	20.47 ± 0.11 (100)	21.46 ± 0.10 (100)
Chord distance *(C) (µm)	$6.18 \pm 5.4 \times 10^{-2}$ (100)	$6.33 \pm 5.8 \times 10^{-2}$ (100)
C/D	$0.30 \pm 2.3 \times 10^{-3}$ (100)	$0.30 \pm 2.8 \times 10^{-3}$ (100)
Pore number	39 ± 0.65 (100)	42 ± 0.83 (100)
Pore diameter (µm)	$3.18 \pm 3.6 \times 10^{-2}$ (100)	$3.21 \pm 3.1 \times 10^{-2}$ (100)
Seed size (mm)	$1.51–1.83 \times 1.41–1.69$	$1.87–2.3 \times 1.67–2.03$
Cotyledon leaves length (mm)	5–6.5 (up to 7) (mean 5.9 mm), more or less curved towards the growing axis	9–12 (mean 10.75) bend toward the outside from about one-third length of the tip
Cotyledon leaves (width) (mm)	1.5–2.5 mm	1.5–2 mm
Leaf shape	Linear	Elliptic lanceolate to oblong
Mature leaf length (mm)	9–22	17–41
Leaf width (mm)	2–4	5.5–10
Leaf length/width ratio	4–10.5 (average 7)	2.5–4.8 (average 3.7)
Leaf cross section	Terete	Narrowly oval or narrowly elliptic
Chlorenchyma cell size length/width (µm)	86.4 ± 5.0 (17)/52.4 ± 1.9	107.9 ± 3.6 (18)/$50,3 \pm 1.2$
Diameter of CCC (µm)	29.0 ± 0.56 (27)	31.7 ± 1.3
	Growth Chamber	
Stomatal density (number of stomata mm^{-2}), adaxial/abaxial	38.6 ± 6.0/35.3 ± 4.5 (20)	28.2 ± 4.5/25.1 ± 5.8 (20)
Volume fraction of mesophyll (%)		
fresh leaves	53.7 ± 7.0	25.0 ± 1.1
fixed leaves	47.7 ± 5.3	27.7 ± 1.7
Number of mesophyll cell layers	2–3	1–2 (3)
Total soluble proteins content, (µg/mg FW)	5.03 ± 0.33	1.87 ± 0.06
Chlorophyll $a+b$ content, (µg/mg FW)	0.89 ± 0.02	0.26 ± 0.03
Chlorophyll a content, (µg/mg FW)	0.72 ± 0.02	0.21 ± 0.03
Chlorophyll b content, (µg/mg FW)	0.18 ± 0.01	$0.05 \pm 6 \times 10^{-3}$
Chlorophyll a/b ratio	4.06	4.88

stigmas) 0.3–0.5 mm long, styles sometimes rudimentary (male flowers), not clavate at apex. Anthers 0.62–0.75 mm long. Filaments 0.5–0.6 mm, around a disc connate to perianth and situated opposite to perianth segments. Seeds suborbicular, flat on upper surface, with a slightly prominent projection in the middle, lower surface convex, 1.87–2.3 x 1.67–2.03 mm.

Etymology. The specific epithet of new species refers to its main distribution range around the Persian Gulf (Sinus Persici).

Representative Specimens Examined. IRAN: Baluchestan: Konarak, sea shore, 19 Nov 1990, *H. Akhani 6755*; Goatr, sea shore, 18 Nov 1990, *H. Akhani 6701* (Hb. Akh.). Fars: In salsis ad Niriz et Mahluja 1600 m, Oct 1892, *J. Bornmüller 4209* (G-Barbey-Boiss., LE); ca. 60 km S of Jahrom, between Hud and Koureh village, 800 m, 29 Nov 1987, *Assadi & Akhani 61859* (TARI). Hormozgan: 52 km W of Bandar Abbas towards Bandar Khamir, along Kol River, 27°3'57"N, 55°47'21"E, 18 m, 20 Dec 2001, *H. Akhani 15988* (Hb. Akh.); 44 km to

$\rightarrow$

FIG. 1. Comparison of *Bienertia sinuspersici* and *B. cycloptera*. A–C. Habit (Khuzestan Province: 17 km N of Bandare Mahshahr)/vegetative branch (Hormozgan Province: 53 km W of Bandar Abbas, Kol River)/flowering branch of *B. sinuspersici*; D–F. Habit (Khorassan Province: Daqe Petergan, 150 km from Qaen, close to Afghanistan border)/vegetative branch (Tehran:

40 km W of Tehran, Mardabad saline flats) /flowering branch of *B. cycloptera*. G, H. Branches/leaves of the two species grown in controlled environment chamber; right *B. cycloptera*, left *B. sinuspersici*. Scale bars = 1.5 cm for G and 1 cm for H. I. Seedlings with cotyledon leaves of the two species grown in the field. Note that *B. sinuspersici* was cultivated in the natural habitat of *B. cycloptera*. J, K. Seeds of *B. sinuspersici*/*B. cycloptera*. Scale bars = 200 μm. Flowering and early fruiting perianth of *B. sinuspersici*. Scale bars = 400 μm for L and 600 μm for M. N, O. SEM of pollen grain of *B. sinuspersici* (images of pollen grains of *B. cycloptera* were published in Akhani et al. 2003). Scale bars = 2 μm for N and 1 μm for O. P. *B. sinuspersici* seeds were collected from native habitat in S Iran, and plants grown in N Iran, native habitat for *B. cycloptera*.

Bandare Charak, on the road from Bandare Lengeh, sea level, 1 Dec 1987, *Assadi & Akhani 61986* (TARI). Kerman: Kahnuj, Kavire-Jazmurian, between Ghaleh Ganji and Chah Reza, 400 m, 8 Nov 1976, *Ershad, Moussavi & Tehrani 40093-E* (IRAN). Khuzestan: NW of Ahvaz, near Karkheh Dam, salty clay soils, 17 Nov 1959, *Pabot* 2580 (G); 35 km W of Ahvaz on the road towards Bandare Mahshahr, 31°14′36″N, 49°3′22″E, 31 Oct 2003, 34 m, *H. Akhani 17424* (Hb. Akh.), 17 km N of Bandare Mahshahr, 30°39′5″ N, 49°15′51″E, 28 m, 31 Oct 2003, *H. Akhani 17433* (IRAN, Hb. Akh.). Ahvaz: near steel mill, 3 Oct 2003, *M. Ghobadnejhad, s.n.* (Hb. Akh.).

IRAQ: Saline mud, W shore of Bahr al-Milh, N of Shithatha, in Karbala Liwa, 21 Nov 1956, *K. H. Rechinger, E. Guest & A. Rawi 28* (G). Distr. Karbala, desertum occidentale, ad litus australe lacus Bahr al-Milh, E Shithata, in limosis salsis, 20 m, 18–20 Nov 1956, *K. H. Rechinger* 8333 (G). Euphrates, near Küt, 11 Dec 1889, Post 18 (G-Barbey-Boiss.).

UNITED ARAB EMIRATES: Abu Dhabi, shores of the Persian Gulf, on saline and sandy soils, sea level, 17 Dec 1990, *H. Akhani 9186* (Hb. Akh.).

Discussion

Morphological and Phenological Features. In spite of several distinct differences that separate *B. sinuspersici* from *B. cycloptera* (Table 1), the species was overlooked by the authors of recent standard floras (Bolous 1996; Hedge et al. 1997) because they studied mostly herbarium specimens and rarely studied the species *in situ*. The extremely succulent nature of *Bienertia* (similar to some other Chenopodiaceae genera such as *Suaeda*, *Salicornia*, and *Climacoptera*) causes loss of important taxonomic characters when dried for herbarium specimens. With the ease of digital photography of fresh specimens, detailed comparisons can be made through examining many pictures. The most distinctive features are the flat and much wider leaves in *B. sinuspersici* versus the (semi-) cylindrical leaves in *B. cycloptera* (Table 1; Fig. 1A–I). The length/width ratio in *B. cycloptera* is nearly twice that of *B. sinuspersici*. Based on the extensive field observations over a range of habitats for both species, it was established that this character is constant and was not influenced by elevation or other ecological conditions. *Bienertia sinuspersici* maintained its leaf morphology when collected from a native habitat in southern Iran and then grown in the same habitat occupied by *B. cycloptera* in central Iran (Fig. 1P). Also, from analysis of plants under controlled growth conditions, the volume of mesophyll/total leaf volume, the soluble protein fresh wt^{-1}, and the chlorophyll content fresh wt^{-1} was about 2 to 3 times higher in *B. cycloptera* than in *B. sinuspersici*, and *B. cycloptera* had a lower leaf chlorophyll *a/b* ratio (Table 1). Another very important distinguishing character

supporting the identity of this new species is the longer cotyledons in *B. sinuspersici*. Furthermore, the cotyledons in *B. sinuspersici* bend toward the outside from about one-third of the length from the tip, but in *B. cycloptera* they are more or less curved towards the growing axis and overlaid by leaves (Table 1; Fig. 1I). The difference in cotyledon leaves is significant taxonomically because of the conservative nature of seedling characters.

The seeds of *B. sinuspersici* are larger than those of *B. cycloptera* (Table 1; Fig. 1J, K). We observed remarkable differences in germination rate and viability of seeds. Two years after collection of *B. sinuspersici* from southern Iran, 80–100% of seeds were viable without any special treatment. However, the seeds of *B. cycloptera* collected from central Iran need a cold treatment before germination, and even then only 10–20% of the seeds germinate. Both species germinate during March–April, but there is an important phenological difference is flowering time. Most populations of *B. cycloptera* in central Iran initiate flowering in July–August and develop fruit from September onwards. The flowering time in *B. sinuspersici* begins as late as October and the fruits begin developing from November onwards. Based on experience in co-cultivating the two species, the difference in flowering time appears genetically fixed rather than determined by different climatic regimes.

Recently Tzvelev (1993) transferred *Suaeda kossinskyi* Iljin into the genus *Bienertia* and provided a new combination, *B. kossinkyi* (Iljin) Tzvelev, because of fused and winged perianth segments. This is a Central Asian and East Caspian species belonging to *Suaeda* Sect. *Brezia* (Moq) Volk that is very close to *S. crassifolia* Pavl. and *S. heterophylla* (Kar. et Kir.) Bunge (Grubov 2000). This complex is composed of C$_3$ hygro-halophytic species that have a horizontal perianth outgrowth that sometimes shows similarities with the perianths of *Bienertia*. However, the inflorescence structure and leaf anatomy do not approach that in genus *Bienertia* (see Freitag and Stichler 2002; Schütze et al. 2003).

Pollen Grains. The pollen grains of *B. sinuspersici* (Fig. 1N,O) are similar to those of *B. cycloptera* (see Akhani et al. 2003, Fig 5A). The mean and standard error of pollen diameter chord distance (distance between the centers of two adjacent pores) and the number of pores per grain in *B. sinuspersici* appear slightly higher than those of *B. cycloptera*, although not significant considering the size of the standard deviations (Table 1). The results indicate pollen characters are a reliable and constant character within the genus *Bienertia* (see Akhani et al. 2003). Besides the use of pollen characters in species identification, the characterization of pollen grains in Chenopodiaceae could help in palynological studies (for example, to determine the
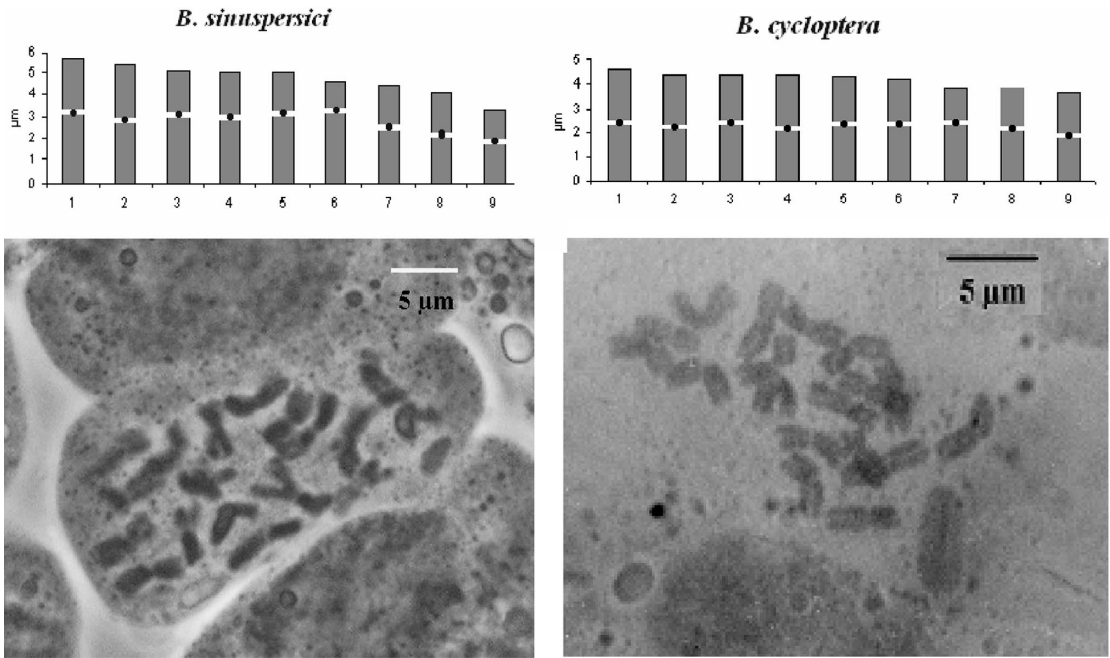
FIG. 2.   Comparison of karyotypes of the two *Bienertia* species.

genus and species in pollen diagrams of sediments in dry habitats).

Cytology.   The results of chromosome studies on somatic cells of *B. sinuspersici* and *B. cycloptera* give chromosome complements of $2n=18$ (Fig. 2). Previously, there have been reports of chromosome numbers for the new species under the name of *B. cycloptera* from Saudi Arabia, Pakistan, and Iraq (Murin and Chaudhri 1970; Al-Turki et al. 2000), which agree with our results. However, the correct and first report of chromosome numbers for *B. cycloptera* is given here. Considering the chromosome numbers, the Chenopodiaceae are rather homogenous with a basic number of $x = 9$, except for *Camphorosma* and *Spinacia*, which have $x=6$ (Turner 1994). Polyploidy is a common phenomenon in this family, particularly in the genera *Suaeda* and *Salicornia* (Ebrahimzadeh et al. 1994).

Karyotypes in both species were symmetric and consisted of eight pairs of metacentric chromosomes and one pair of submetacentric chromosomes (Table 2; Fig. 2). Comparison of the two karyotypes shows clear differences between both species including the length of arms, the arm ratio and total length of chromosome complement (Table 2). There are also differences in the position of the submetacentric chromosome. Therefore, the cytological data are in accordance with other evidence for two separate *Bienertia* species. A comparison with the data obtained from chromosome analysis in this species and other genera of Chenopodiaceae, like *Suaeda* (Ebrahimzadeh et al. 1994) and several genera of subfamily Salicornioideae (S. M. Ghaffari, L. Seyedrasi and H. Akhani [unpubl. data]), shows that the chromosomes of *Bienertia* are more symmetrical than in many other studied genera within Chenopodiaceae.

TABLE 2.   The length of chromosomes (μm) in two *Bienertia* species at metaphase of mitotic division. L = long arm, S = short arm, m = metacentric, sm = submetacentric.

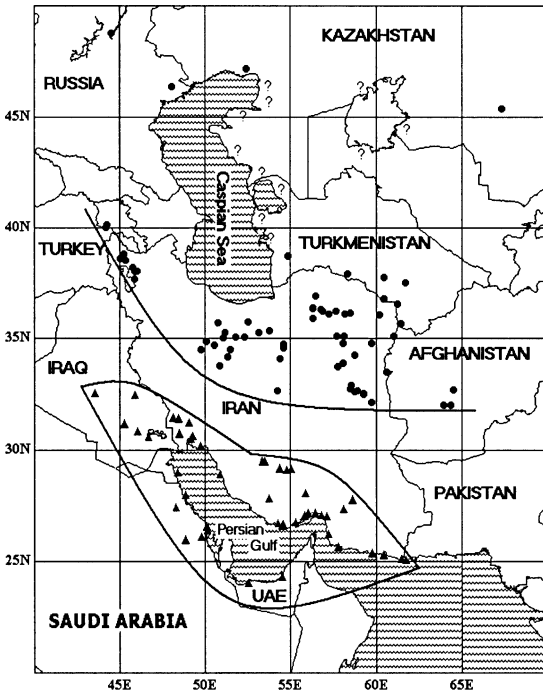|	B. sinuspersici					B. cycloptera				
Chromosome #	L	S	L + S	L/S	Type	L	S	L + S	L/S	Type
1	3.16	2.55	5.71	1.24	m	2.55	2.15	4.70	1.18	m
2	2.80	2.70	5.50	1.03	m	2.32	2.12	4.44	1.09	m
3	3.02	2.05	10.57	1.47	m	2.49	1.9	4.39	1,31	m
4	2,81	2.20	4.81	1.27	m	2.24	2.14	4.38	1.04	m
5	3.07	1.94	5.01	1.58	m	2.65	1.73	4.38	1.53	m
6	3.19	1.41	4.60	2.26	sm	2.44	1.81	4.25	1.34	m
7	2.43	1.98	4.41	1.22	m	2.47	1.44	3.91	1.71	sm
8	2.17	1.88	4.05	1.15	m	2.23	1.64	3.87	1.35	m
9	1.78	1.52	3.30	1.17	m	1.87	1.86	3.73	1.00	m

FIG. 3. Distribution map of *Bienertia sinuspersici* (triangle) and *B. cycloptera* (dot), modified from Akhani et al. 2003.

As hypothesized by Stebbins (1971), symmetrical karyotypes indicate plesiomorphic character. Therefore, the chromosome data are in accordance with other studies indicating *Bienertia* is an isolated, and probably an older, genus in Chenopodiaceae (Akhani et al. 2003; Schütze et al. 2003).

Distribution and Phytogeography. The range of *B. sinuspersici* starts from the westernmost coasts of Pakistan (25°07′N, 61°30′E) and extends westward all along the coastal areas in southern Iran (Baluchestan, Hormozgan, Fars, Bushehr, and Khuzestan Provinces) and countries surrounding the Persian Gulf, including the United Arab Emirates, northern Saudi Arabia, Qatar, Kuwait, and Iraq (around Bahr al Milh, 32°34′N, 43°30′E). It shows an arc-like, latitudinal range that is separated from the range of *B. cycloptera* populations by the Zagros and Makran Mountains (Fig. 3). Although it was not possible to check all reported plants in various herbaria, according to the material examined, the new species is not confined to the lower elevations of the coastal zones. It occurs also around inland saline areas in the Fars province at elevations as high as 1600 m. Before recognizing the novelty of *B. sinuspersici*, there was an effort to interpret the unusually wide latitudinal range of what was considered to be a single species of *Bienertia* (including Freitag 1991). However, it has become evident that this range is not that of a single species, but of two species.

The range of *B. sinuspersici* is a natural biogeograph-

ic range that has been interpreted differently by various biogeographers. According to Zohary (1973), except for the westernmost parts of the area in the Khuzestan province in Iran and the Iraqi lowlands, which belong to the Saharo-Arabian region, the remaining parts lie in the Nubo-Sindian province of the Sudanian region. Léonard (1989) and White and Léonard (1991) considered southern Iran to be part of the Saharo-Sindian regional zone, which belongs to the Nubo-Sindian local center of endemism. The discovery of this new species, and some other undescribed new species (in preparation), and evaluation of data from other genera of Chenopodiaceae and several other families has led us to emphasize the role of the Irano-Turanian endemic species in southern Iran (Akhani 2004). The range of several other C_4 chenopods like *Halanthium purpureum* (Moq.) Bunge, *Salsola drummondii* Ulbr., *S. griffithii* (Bunge) Freitag & Khani, *Suaeda baluchestanica* Akhani & Podl., *Halothamnus iranicus* Botsch., and many other species belonging to other families, lie in this biogeographic zone (Rechinger 1963–2001).

Leaf Morphology and Anatomy. Both in cylindrical leaves of *B. cycloptera* and in flattened leaves of *B. sinuspersici*, epidermal cells are more elongated on the adaxial side of the leaf (Fig. 4A, D) in comparison with a mostly polygonal shape on the abaxial side (Fig. 4B, E). The leaves are amphistomatic (approx. the same number of stomata on both sides of the leaf) in each species (Table 1). With plants grown under the same conditions in controlled environments, *B. sinuspersici* had somewhat lower stomatal density than *B. cycloptera*. The sunken stomata are mostly of the anomocytic type without any specific difference in the surrounding epidermal cells; but, sometimes one of the epidermal cells is smaller than the others (Fig. 4A,B,D,E). Comparison of epidermal cell and guard cell sizes did not reveal any significant difference between the two species (results not shown).

The epidermis, and even very young branches, are covered by bladder-like stalked hairs or trichomes (Fig. 4C, F). In both species they are more frequent on the abaxial epidermis, with a sparser distribution on the adaxial side of the leaf of *B. sinuspersici*, and they are nearly absent on the adaxial side of *B. cycloptera* leaves. In nature these hairs usually are persistent until flowering but they can persist longer in growth chambers. Superficially, these hairs are similar to those in *Atriplex* (Fahn 1974; Thomson 1975) where they were designated as vesiculated hairs (trichomes).

The leaf anatomy of *B. sinuspersici* is similar to *B. cycloptera* in terms of the absence of Kranz anatomy and the presence of a novel central cytoplasmic compartment (CCC) in the chlorenchyma cells (Freitag and Stichler 2002; Voznesenskaya et al. 2002). A difference exists in *B. cycloptera* having two to three layers of elongated chlorenchyma cells around the cylindrical leaf
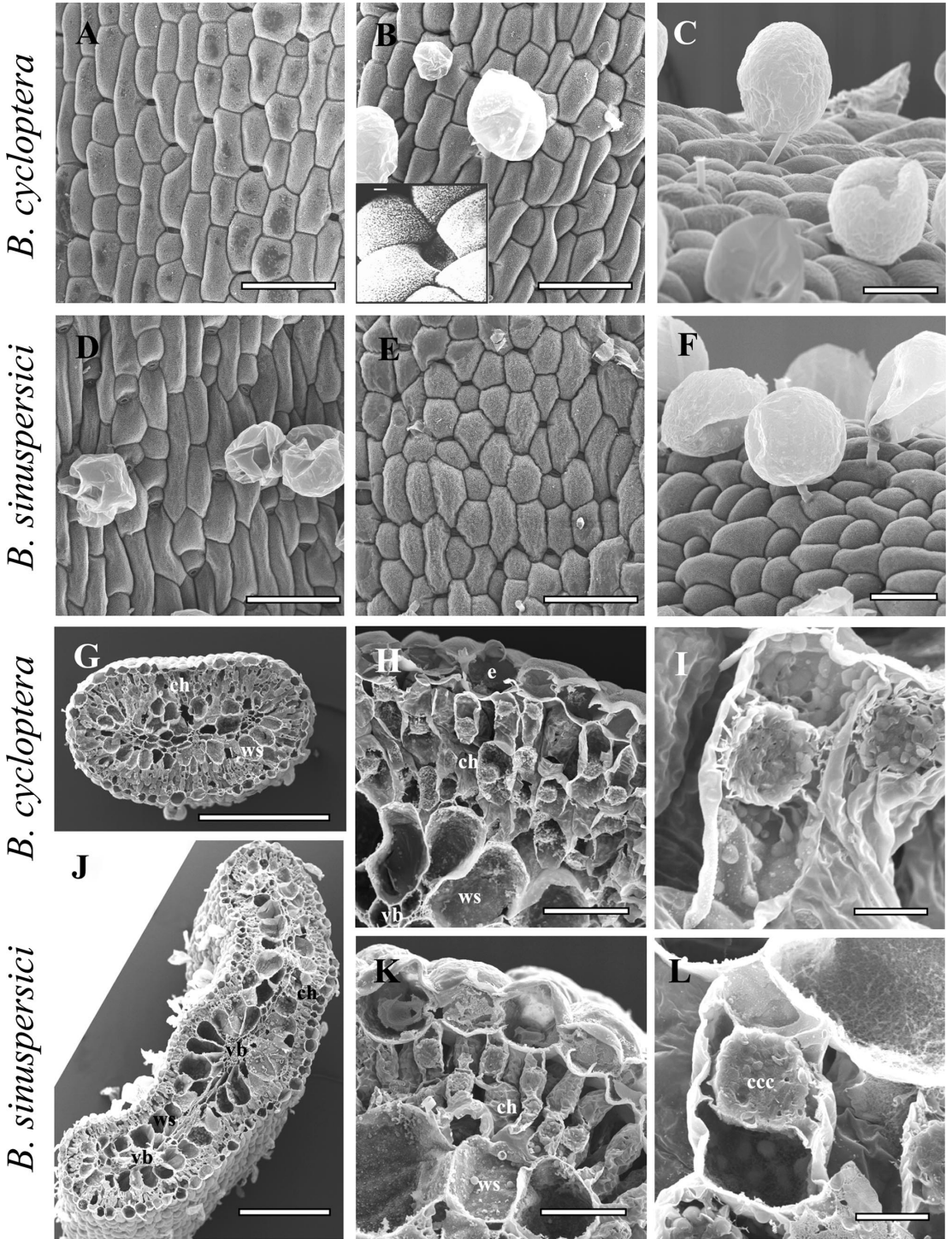
FIG. 4. Comparison of epidermal characteristics (A–F), general leaf anatomy (G, J), and chlorenchyma structure (H–L) for two species of *Bienertia* using scanning electron microscopy. A–C. Epidermal surface of *Bienertia cycloptera*: adaxial (A), abaxial (B) with inset showing the sunken stomata, and trichomes on stalks (C). D–F. Epidermal surface of *Bienertia sinuspersici*: adaxial (D), abaxial (E), and trichomes on stalks (F). G–L. Leaf cross sections of *Bienertia cycloptera* (G–I) and *Bienertia sinuspersici* (J–L). The size of *Bienertia sinuspersici* leaf is much larger than in *B. cycloptera*. Scale bars = 1 mm for G, J and 20 μm for all others. Labels: ccc, central cytoplasmic compartment; ch, chlorenchyma cells; e, epidermis; vb, vascular bundle; ws, water storage tissue.
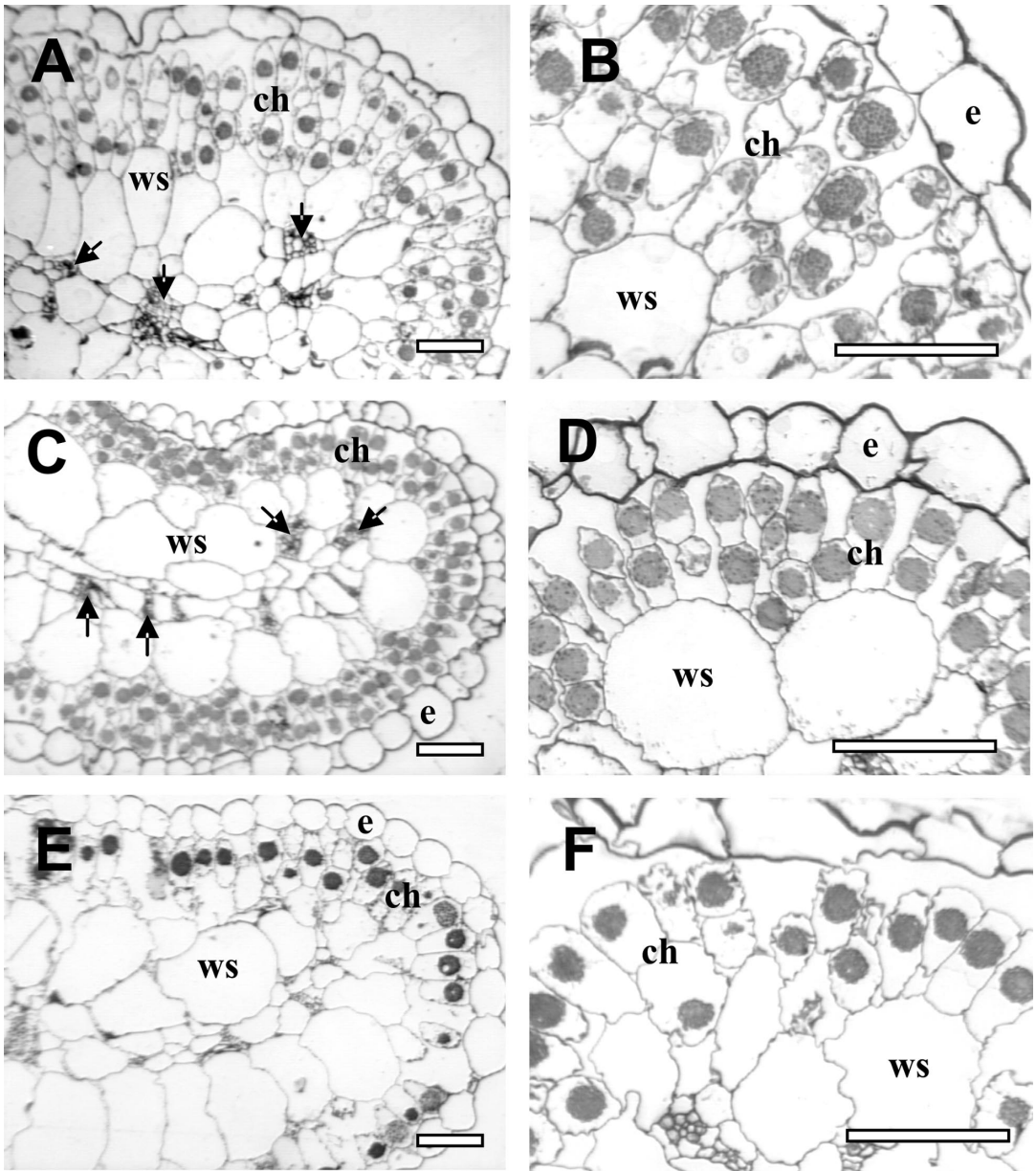
FIG. 5.   Light microscopy micrographs of cross sections of mature leaves of two *Bienertia* species. A, B. *Bienertia cycloptera*. A. General structure of the leaf showing several layers (2–3) of chlorenchyma, and vascular bundles (arrows) surrounded by water storage tissue. The main bundle is in the middle of the leaf and lateral vascular bundles occur in an arc from the central bundle to the edge of the leaf. B. High magnification of leaf segment showing the presence of two to three layers of chlorenchyma cells with a large central cytoplasmic compartment. C–F. *Bienertia sinuspersici*. C, E. General structure of the leaf showing position of vascular bundles (arrows). There is variation in the number of chlorenchyma cell layers from mostly two in some plants (C, D) to mostly one in other plants (E–F). D, F. Structure of chlorenchyma cells at higher magnification, showing the large central cytoplasmic compartment. Scale bars = 100 μm. Labels: ch, chlorenchyma cells; e, epidermis; vb, vascular bundle (indicated by arrows); ws, water storage tissue.

(Figs. 4G-I, 5A,B), compared to *B. sinuspersici* having one to two layers of chlorenchyma in flattened leaves (Fig. 4J-L, 5C-F). The chlorenchyma cells are ellipsoid, with very extensively developed intercellular spaces. The whole structure, as well as the appearance and

size of chlorenchyma cells and the CCC, is very similar in both species (Table 1). The water storage cells in both species are usually represented by one layer of large cells and also, sometimes, with an additional layer of small cells around the vascular bundles (Fig. 5).

TABLE 3. Comparison of δ^{13}C of *Bienertia sinuspersici* and *Bienertia cycloptera* from different origins and with different collection dates. The literature records from Saudi Arabia and Iraq have been reported previously under *B. cycloptera,* but are given here under *B. sinuspersici*. Samples are from field grown plants, or where indicated from mature leaves of growth chamber or greenhouse grown plants. * 5 replicates. Kavire-Mobarakieh is located at the margin of Kavir Protected Area (35°4′N, 51°47′E, 823m), and Mardabad is located 40 km West of Tehran (35°42′N, 50°45′E, 1164m).

Species, Part	δ^{13}C	Locality and voucher	Dates or references
Bienertia sinuspersici			
Leaf	−13.9	S. Iran, Hormozgan, Akhani 15988	20.12.2001
Fruiting perianth	−14.4	S. Iran, Hormozgan, Akhani 15988	20.12.2001
Leaf	−13.4	Pakistan: Baluchestan	(Freitag and Stichler 2002)
? Leaf	−15.4	Saudi Arabia	(Winter 1981)
? Leaf	−13.8	Iraq: Rawi & Serhabriel 6300	(Voznesenskaya et al. 2002)
? Leaf	−14.5	Iraq: Khatib & Alizzi 32648	(Voznesenskaya et al. 2002)
? Leaf	−11.5	Iraq: Winter, Hilly & Mousa-wi 31.10.1974.	(Voznesenskaya et al. 2002)
Mature 2–3 cm leaves, green-house	−14.6*	Origin: Iran: Hormozgan	—
Mature leaves, greenhouse	−15.3	Origin: Iran: Hormozgan	—
Bienertia cycloptera			
Leaves	−15.5	Origin Iran: Kavire Moba-rakieh	(Freitag and Stichler 2002)
Leaves	−15.6	Mardabad	9.8.02
Leaves	−14.2	Mardabad	13.8.02
Leaves	−14.1	Arak, 4 km S of Anjilavand, Akhani 1170	17.9.1986
Stem	−13.1	Mardabad, Akhani 16477	11.10.02
Leaves	−13.1	Mardabad, Rude-shur	11.10.02
Fruiting flowers	−14.7	Mardabad	11.10.02
Roots	−12.8	Mardabad	11.10.02
Roots	−12.7	Kavire-Mobarakieh, Akhani 16485	17.10.02
Stems	−13.6	Kavire-Mobarakieh	17.10.02
Flowers	−14.8	Kavire-Mobarakieh	17.10.02
Leaves	−12.6	Kavire-Mobarakieh	17.10.02
Stems	−14.8	Mardabad	10.11.02
Flowers	−15.5	Mardabad	10.11.02
Leaves	−13.1	Mardabad	10.11.02
Roots	−12.4	Mardabad	10.11.02
Leaves Growth chamber	−13.6	Kavire-Mobarakieh	(Voznesenskaya et al. 2002)

The vascular bundles are arranged in the lateral longitudinal plane with a main, large central vascular bundle and several lateral ones. The number of small lateral bundles is higher in the flat leaves of *B. sinuspersici* than in the cylindrical leaves of *B. cycloptera*; nevertheless, in both species one or two of the most lateral bundles are turned with their phloem facing to the adaxial side of the leaf (Fig. 5A, C). In general, the arrangement of vascular bundles is similar to that in representatives of the genus *Suaeda*, except for the presence of turned lateral bundles (Carolin et al. 1975; Schütze et al. 2003). In both species the anatomical features of cotyledons are similar to those in leaves of the corresponding species (not shown).

These *Bienertia* species are desert plants. All desert plants have a set of common physiological and morphological features and they have been classified as eremophytes (Nikitin 1966). The anatomy and mor-

phology of plants highly depends on the type of desert (Vassilevskaya 1940); but most desert plants, as well as xerophytes in general, are characterized by a reduction in the size of the above ground structure, reduction of leaf surface, and the presence of a high density of trichomes on the leaf surface (Eberhardt 1903; Radkevitch and Vassilevskaya 1933; Vassilevskaya 1940). Both *Bienertia* species are different from most desert plants in that they are capable of forming large bushes during one growing season, and in having a loose distribution of large chlorenchyma cells in the leaves, which is more characteristic of halophytes (Caldwell 1974). Nevertheless, they have succulent leaves with well-developed water storage tissue and sunken stomata, which are common features of plants of Central Asian deserts and halophytes (Vassilevskaya 1940).

The evolutionary and adaptive significance of flat leaves in *B. sinuspersici* versus the cylindrical leaves in

B. cycloptera may be related to macro-climatic differences rather than local and edaphic conditions. *Bienertia cycloptera* occurs in temperate and cold deserts of central Iran, Afghanistan, the Armeno-Turkish area, and northern and eastern parts of the Caspian Sea with extremely dry and continental climate. *Bienertia sinuspersici*, in contrast, occurs in areas having a hot climate, and where the humidity of the air is very high because of the sea (Fig. 3). Interestingly, we find that *Suaeda baluchestanica* Akhani & Podlech from the coasts of the Oman Sea in southeast Iran and in Pakistan has flat leaves similar to *B. sinuspersici*. In this species, the leaves, like those of *B. sinuspersici*, are much flatter and larger than the inland species *S. fruticosa* (Akhani and Podlech 1997).

Carbon Isotope Composition. Carbon isotope values for *B. sinuspersici* and *B. cycloptera* from a range of natural sites and from greenhouse and growth chamber grown plants from previous and current analyses are shown in Table 3. In *B. sinuspersici* the values ranged from -11.5 to -15.4‰ with a mean $\delta^{13}C$ value of -14.1‰ for 9 measurements; in *B. cycloptera* the values ranged from -12.4 to -15.6‰ with a mean $\delta^{13}C$ value of -13.9‰ from 17 measurements. The results show that both species have C_4 type carbon isotope composition.

In summary, *Bienertia sinusperisci* has a range of features that distinguishes it as a distinct species from *B. cycloptera*, and thus we describe it as a new species within the genus *Bienertia*. In the future, we need to explore three important aspects of this group: the putative origin and dispersal of the two species of *Bienertia*; clarification of the ambiguous position of the genus between subfamilies Suaedoideae and Salicornioideae; and the genetic basis and evolution of C_4 photosynthesis in a single cell in this genus. Among the interesting questions are: did these species evolve by normal vicariant events from a common distribution in the past? Is one species ancestral and the other evolved from a secondary introduction into new habitats? If so, which is ancestral and which is derived? These questions need to be considered, along with the broader context involving several other sister species, such as *Suaeda arcuata* Bunge and *S. aegyptiaca* (Hasselq.) Zohary, *Heliotropium aucheri* DC. and *H. digynum* (Forssk.) C. Christ. (Akhani and Förther 1994), and *Bassia eriantha* (Fisch. & C. A. Mey.) O. Kuntze and *B. eriophora* (Schrader) Aschers.

Both *B. cycloptera* and *B. sinuspersici* have the single-celled C_4 photosynthesis system, despite the fact that their habitats are different. These unique plants will be of interest to plant biologists interested in evolution and mechanisms of photosynthesis in terrestrial plants, specialized adaptations to stressful environments, and biogeography and speciation. This discovery is not only important for those scientists who are interested in introducing C_4 photosynthesis into C_3 plants through genetic engineering—a task which is considered very difficult because of the complex structure of Kranz anatomy—but it also shows that this novel strategy for CO_2 fixation is not restricted to only two species. We stress hereby the importance of genetic diversity in the Middle East and in particular, in Iran. The present land use, and environmental protection policy of the region may cause extinction of valuable and unique genetic diversity before science reveals their existence. Several of these examples have been given by Akhani (in press).

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