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From the frying pan: an unusual dwarf shrub from Namibia turns out to be a new brassicalean family

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Abstract

Tiganophyton karasense, an evergreen dwarf shrub, is described as a new species. A new genus and family are also proposed for it in the order Brassicales. Phylogenetic analysis of DNA sequence data indicate that *Tiganophyton* is sister to Bataceae/Salvadoraceae, and all three sister to Koeberliniaceae. First realized to be undescribed in 2010, *T. karasense* is a rare species known only from three localities in the arid Karas Region, southern Namibia. These small shrubs grow near the edges of seasonal pans on calcareous substrate underlaid by shales and mudstones of the Prince Albert Formation of the Karoo Supergroup. Morphological characters diagnostic of the new family include: a marked differentiation into long and short shoots; dimorphic, spirally arranged leaves; glucosinolate production; bisexual laterally flattened flowers borne singly in bract axils on short shoots only; tetramerous calyx, corolla and androecium with fused sepals and free, non-clawed petals; a staminal disc, but no nectary glands; deeply bilobed ovary with a gynobasic style; S-shaped gynophore supporting a bilocular, horizontally orientated or inverted ovary; two ovules per locule; and a dry, persistent fruit, provisionally interpreted as a one-seeded nutlet. Based on IUCN Red List categories and criteria, a conservation assessment of Vulnerable (VU D1) is recommended for *Tiganophyton karasense*.

Keywords: Bataceae, Brassicales, Koeberliniaceae, limestone, mudstone, seasonal pans, Karoo Supergroup, endemism, Karas Region, Salvadoraceae, taxonomy, Tiganophytaceae, *Tiganophyton*

Introduction

During a botanical excursion to the arid Karas Region of southern Namibia in January 2010, one of us (WS) encountered an unfamiliar dwarf shrub with tightly arranged dimorphic leaves borne on distinct long and short shoots. Although seemingly sterile at the time, the initial impression was that it might be a member of the amaranthaceous genus *Salsola* Linnaeus (1753: 222), which is common and diverse in arid parts of Namibia. A follow-up visit in December 2010 found the plants in flower and fruit, enabling fertile material to be collected. The morphology of flowers and fruit indicated that it belongs neither to *Salsola* nor Amaranthaceae. Based on morphology, relationships of the taxon were not immediately obvious. To establish the possible phylogenetic position of our mystery plant, the plastid *rbcL* gene was sequenced. A preliminary phylogenetic analysis of this molecular data unexpectedly placed the taxon in order Brassicales *sensu* APG IV (Angiosperm Phylogeny Group IV 2016).

The suggested position of the new taxon in Brassicales was subsequently supported by an assessment of its floral morphology, confirmation of glucosinolate production and analyses of three additional coding regions, two

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plastid (*ndhF* and *matK*) and one mitochondrial (*matR*). However, the new taxon differs from closely related families in several morphological features. Hence, here this dwarf shrub is formally described as a new species, genus and family, namely *Tiganophyton karasense* and Tiganophytaceae, endemic to the Karas Region of southern Namibia. Examination of the *Salsola* holdings in PRE and WIND revealed one earlier collection of the new species (*Strohbach 3636*), filed under *Salsola tuberculata.*

Materials and methods

Field observations, comparative morphology and mapping

Morphological descriptions and ecological information presented here are based on field observations and material collected by one of us (WS) following extensive field work in Namibia. This was supplemented by study of relevant literature and herbarium collections.

The following herbaria were consulted for possible collections of the new species that might have been filed under *Salsola*, a superficially similar member of Amaranthaceae: PRE, PRU and WIND (herbarium acronyms follow Thiers 2019). Locality information for specimens cited also provide the particular quarter degree grid squares following the degree reference system of Edwards & Leistner (1971).

The distribution map was compiled from specimen data using ArcView 3.1 software. The original base map is based on the GTOPO30 global digital elevation model. The colours were modified in Global Mapper v6.06. Conservation assessment follows IUCN (2012).

Phylogenetic analysis

To place *Tiganophyton* within the flowering plants, we first produced a sequence of the plastid gene *rbcL* from material collected in the wild (*Swanepoel 364*, WIND). DNA was extracted using a modified version of the 2× CTAB method (Doyle & Doyle 1987). Amplification used standard primers (i.e. Olmstead *et al.* 1992) and the ReddyMix PCR Master Mix from ABgene (2.5 mM MgCl2; Epsom, Surrey, UK) with the addition of 1 µl of bovine serum albumin 0.4% and 50 ng of each primer, in a final volume of 25 µl. Amplifications were performed as follows: 2 min initial denaturation at 94 °C, followed by 32 cycles of 1 min denaturation at 94 °C, 1 min annealing at 48 °C, 1.5 min extension at 72 °C, and a final extension of 3 min at 72 °C. PCR products were purified using the Nucleospin Extract II kit (Machery-Nagel, Düren, Germany). Cycle sequencing reactions were conducted in 10 µl reactions using 1 µl of BigDye® Terminator cycle sequencing chemistry (v3.1; ABI; Warrington, Cheshire, UK) and run on a ABI 3730 automated sequencer. Complementary strands were assembled in Geneious (version 8.1.9; Kearse *et al.* 2012). The resulting *rbcL* sequence (GenBank accession MN628298) was incorporated in the large eudicot *rbcL* phylogenetic analysis of Savolainen *et al.* (2000) using the MUSCLE algorithm (Edgar 2004) as implemented in Geneious. Three additional species belonging to Salvadoraceae, identified as close relatives of *Tiganophyton* in a preliminary phylogenetic analysis, were also included in the phylogenetic analysis: *Azima tetracantha* Lamarck (1783: 343**)** (GenBank accession U36782), *Salvadora angustifolia* Turrill (1918: 202) (U38532) and *Salvadora australis* Schweickerdt in Obermeyer *et al.* (1937: 248) (JF265581).

To strengthen the reliability of the position of *Tiganophyton* within Brassicales obtained from the *rbcL* analysis and increase support for a position near Salvadoraceae and Bataceae, we added sequences of *Tiganophyton* (plastid *ndhF* and *matK*, mitochondrial *matR*) to the phylogenetic analysis of Brassicales conducted by Cardinal-McTeague *et al.* (2016) (GenBank accessions MT165894, MT165893 and MT165892). These sequences were obtained from the off-target reads of material of *Tiganophyton* processed using a targeted enrichment approach as described in Johnson *et al.* (2019). We also included in this multi-gene matrix the *rbcL* sequences used in the previous analysis for *Salvadora* Garcin ex Linnaeus (1753: 122) and *Azima* Lamarck (1783: 343), which were not included in Cardinal-McTeague *et al.* (2016).

For both analyses, the maximum likelihood criterion as implemented in the software RAxML (v. 8.2.12; Stamatakis 2014) on the CIPRES portal (www.phylo.org) was used to reconstruct a phylogenetic tree. The analysis was performed with the GTRCAT model default parameters. Rapid bootstrap replicates (1000) were followed by a maximum likelihood analysis.

Chemical analysis

A sample of air-dried material (stems with leaves) of the new species (*Swanepoel 364*, WIND) was analysed for the presence of glucosinolates by liquid chromatography-mass spectrometry (LC-MS) at the Mass Spectrometry Unit, Central Analytical Facilities, University of Stellenbosch, South Africa. The sample was extracted with 70% methanol overnight, and analyses were performed on a Waters HSS T3, 2.1 × 150 mm column using the same conditions as Stander *et al.* (2017). In short, a 0.1% formic acid to acetonitrile gradient was applied, and data were acquired using electrospray ionization in the negative mode on a Waters Synapt G2 quadrupole-time-of-flight mass spectrometer and Waters Acquity ultra-high-pressure liquid chromatograph (UPLC). The instrument was calibrated with sodium formate, and leucine encephalin was used as lock mass for accurate mass determinations. Sinigrin hydrate ($C_{10}H_{16}KNO_9S_2 \cdot xH_2O$; Supelco product 0290) was used as a glucosinolate reference standard to confirm the presence of sinigrin. The other glucosinolates were identified based on their elemental composition and MS fragmentation.

Results

Phylogenetic analysis

In the resulting tree based on *rbcL* alone (not shown), *Tiganophyton* falls in Brassicales as sister to a paraphyletic Salvadoraceae and Bataceae. The second analysis focussing on Brassicales produced identical results (Fig. 1), although with stronger bootstrap support for the relationship between *Azima* and *Batis* Browne (1756: 357) and within *Salvadora*. Although *Salvadora* is sister to *Azima*, both Salvadoraceae, plus *Batis* (Bataceae), we cannot draw firm conclusions regarding relationships of Salvadoraceae and Bataceae, because *Dobera* Jussieu (1789: 425) (Salvadoraceae) is missing, and not all species of *Azima* and *Batis* are included. However, this position of *Tiganophyton* is highly supported (100%) in both analyses.

Chemical analysis

The results of the LC-MS analysis of a methanolic extract of *Tiganophyton karasense* show the presence of sulphate groups and nitrogen, the combination of which has been taken as signalling the presence of glucosinolates. However, no glucosinolate similar to sinigrin hydrate (the reference compound) is present, and hence the precise identity of the specific glucosinolates present in the material remains to be elucidated.

Discussion

The considerable value of molecular data for the establishment of family relationships was again clearly demonstrated by our study. In general appearance, *Tiganophyton karasense* is morphologically so strikingly atypical for a member of Brassicales that initially placement in this order was not suspected. Although apparently absent from some Brassicales, glucosinolates are considered one of the key markers for the order (Fay & Christenhusz 2010, Christenhusz *et al*. 2017, Leite & Castilho 2017). Hence confirmation of glucosinolates in *T. karasense* strongly supports its placement within the Brassicales.

Once a position in Brassicales was established, a re-examination of the general morphology of the flowers of the new species provided some support for such a placement. Morphological characters shared by *T. karasense* with many members of the Brassicales include the spirally arranged leaves, bisexual and tetramerous flowers, free petals and, in particular, the presence of a gynophore.

A gyno- or androgynophore has hitherto been reported in at least some members of ten of the 17 families included in Brassicales by APG IV (Angiosperm Phylogeny Group IV 2016), namely (in alphabetical order) Brassicaceae, Capparaceae, Cleomaceae, Emblingiaceae, Koeberliniaceae, Moringaceae, Pentadiplandraceae, Resedaceae, Salvadoraceae (short, *sensu* Ronse De Craene & Wanntorp 2009) and Setchellanthaceae (Stevens 2001 onwards). However, the gynoecium in *T. karasense* is distinct and, as far as we can ascertain, unmatched in Brassicales. The gynophore, often seemingly eccentrically inserted on the staminal disk/receptacle, is curved (roughly S-shaped) so as to place the ovary in a horizontal or inverted position. The ovary itself is deeply bilobed with the style inserted between the lobes. We have provisionally interpreted the style as being gynobasic, known elsewhere in Brassicales only in Limnanthaceae, a small family of annuals from temperate North America.

Based on results of the phylogenetic analysis and considering the unusual morphology of *T. karasense*, we decided to place it in a family of its own. Diagnostic characters for the family are summarized under the formal family description below and selected morphological features of Tiganophytaceae are compared with its possible nearest family relatives, namely Salvadoraceae and Bataceae, but also with Koeberliniaceae, the sister to Tiganophytaceae+Bataceae/Salvadoraceae (Table 1). *Tiganophyton* is morphologically distinct from all three families and an edaphic specialist, which may be of taxonomic significance as an ecological property shared with Bataceae and Salvadoraceae. Bataceae and some members of Salvadoraceae grow in saline soils, whereas *T. karasense* is suspected to be a calcicole, but this needs confirmation. Calcic and saline soils in arid parts of southern Africa are often correlated (e.g. Buch & Rose 1996, Fey 2010).

TABLE 1. Selected features of the newly proposed Tiganophytaceae compared with the most closely related families as suggested by molecular evidence. Information for the other families extracted mainly from Stevens (2001 onwards), Bayer & Appel (2003), Kubitzki (2003a, b), Takhtajan (2009), Christenhusz *et al.* (2017), and Mabberley (2017).

Character	Tiganophytaceae	Bataceae	Salvadoraceae	Koeberliniaceae
Distribution	Small region in southeastern Namibia	Warm-temperate and tropical coasts in the American Atlantic, Central American Pacific, Galapagos and the Timor Sea in Australia and Indonesia	Old World, especially Africa, Madagascar, Arabia and India	Southwestern North America, with disjunct population in Bolivia
Habitat	Hot, arid, calcareous seasonal pans in semi-desert region	Marine habitats: salt marshes, mangroves, beaches, sand bars and coasts	Warm, arid, often on somewhat saline soils	Warm, arid, semideserts and deserts
Habit	Xerophytic dwarf shrubs, up to 0.8 m tall	Succulent shrubs, often scrambling	Shrubs or trees, sometimes scrambling or scandent	Xerophytic shrubs or small trees
Plant sex (*sensu* Bawa 1980)	Hermaphroditic	Monoecious or dioecious	Hermaphroditic or dioecious (*Azima*)	Hermaphroditic
Glucosinolates	Present (presence of myrosin cells still to be confirmed)	Present (myrosin cells present)	Present (myrosin cells absent)	Apparently absent (although myrosin cells present)
Stems	Distinctly differentiated into long and short shoots	Mainly long shoots	Mainly long shoots	Mainly long shoots
Thorns/spines	Stem of weathered old short shoots sometimes persisting as a short thorn or blunt projection	Absent	Absent or present (spines)	Present (thorns)
Foliage leaves				
Duration	Evergreen	Evergreen	Evergreen	Drought-deciduous
Size and texture	Dimorphic; minute, scale-like, leathery or semi-succulent	Monomorphic; succulent, ca. terete; bracts of cone-like spikes scale-like	Monomorphic; leathery or semi-succulent, never scale-like	Monomorphic; minute, scale-like, leathery

......*continued on the next page*

TABLE 1. (Continued)

Character	Tiganophytaceae	Bataceae	Salvadoraceae	Koeberliniaceae
Stipules	Absent	Present (minute, early deciduous)	Present (minute)	Absent
Arrangement	Alternate, spiral, dense	Opposite, well-spaced; bracts decussate, dense	Opposite, well-spaced	Alternate, spiral, well-spaced
Inflorescences	Flowers borne solitarily in axils of bracts on short shoots	Axillary or terminal cone-like spikes	Axillary or terminal racemes, paniculate or axillary fascicles	Short, axillary, umbel-like racemes
Flowers				
Sex	Bisexual	Unisexual	Bisexual or unisexual	Bisexual
Symmetry	Actinomorphic, but laterally compressed	Actinomorphic, laterally compressed (disymmetric)	Actinomorphic, laterally compressed at least in early stages	Actinomorphic
Sepals	4, fused with free lobes	2, fused, split into two lobes in males; absent in females	2–4(–5), fused into a short tube	4, free
Petals	4, free, sessile	4, free, long clawed in males; absent in females	4(–5), basally fused, sessile	4, free, slightly clawed
Stamens	4, free	4, free	4(–5), free or basally fused (sometimes with petals) into a short tube	8(–10), free
Nectar gland (disk-like)	Present (?), 4-lobed, each lobe continuing as a stamen; alternatively interpreted as a receptacle	Absent	Absent	Absent
Nectar glands (separate)	Absent	Absent	Often present, scale-like, ventrally at base of petals	Present, scale-like, at base of stamens
Ovary (form)	Deeply bilobed; bilocular; horizontally orientated, occasionally inverted	Not lobed; tetralocular; upright	Not lobed; uni- or bilocular; upright	Not lobed; bilocular; upright
Gynophore	Present, erect in very young flowers, near S-shaped in mature ones	Absent	Absent, or if present, short and straight	Present, straight
Ovules	2 per locule	1 per locule	1 or 2 per locule	Many per locule
Style	Well developed, elongate, deeply inserted between lobes of ovary (gynobasic)	Short, apical; stigma subsessile	Short, apical	Short, apical
Fruit (type)	Dry, papery (enclosed in persistent calyx); 1-seeded nutlet (?)	Drupe or drupaceous syncarp, each with 4 pyrenes	Drupe or berry; 1-seeded	Berry; 1- or 2-seeded

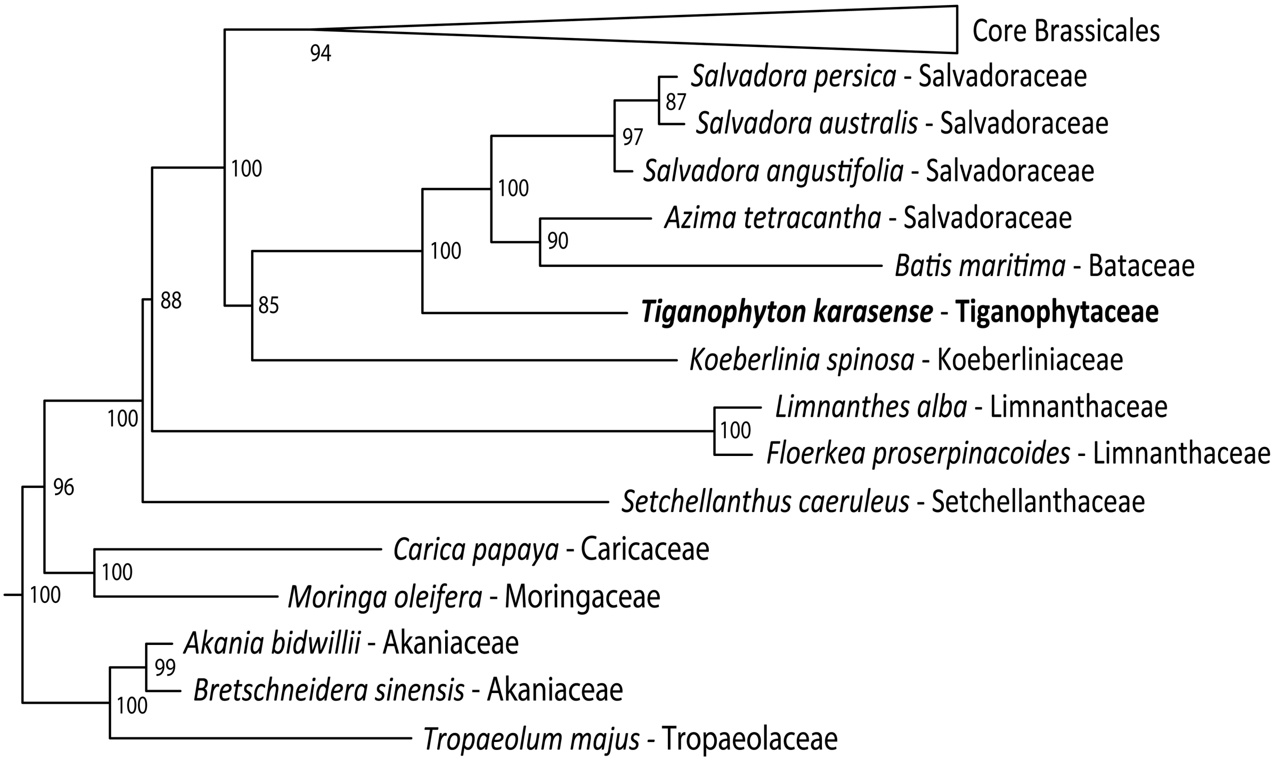

FIGURE 1. Portion of the Brassicales phylogenetic tree based on three plastid (*ndhF*, *matK* and *rbcL*) and one mitochondrial (*matR*) markers. The core Brassicales clades comprise samples from Brassicaceae, Capparaceae, Cleomaceae, Resedaceae, Gyrostemonaceae, Pentadiplandraceae, Tovariaceae, and Emblingiaceae. Numbers on nodes are bootstrap percentages from the RAxML analysis.

We have refrained from attributing too much taxonomic significance to some features of the ovary in *T. karasense*, because of difficulties of interpreting these with confidence. The ovary is small (ca. 0.4 mm diam.) and strongly laterally flattened between the tightly packed leaves and bracts of the short shoots. Although clearly bilobed, it was not possible to establish with certainty under a dissecting microscope whether it is bilocular or unilocular. We have assumed that each lobe of the ovary represents a single locule and hence have provisionally described it as bilocular. Each lobe of the ovary contains two ovules attached basally (at the point of gynophore attachment) in each locule, bearing in mind that the ovary may be inverted. However, details of placentation and ovule type still need to be elucidated. Likewise, we were unable to interpret the precise derivation of the small (0.8–1.2 mm diam.), tough-walled "seeds", which are retained for several seasons between the leaves and bracts of the short shoots. It is not clear whether these structures are derived from the ovule only or ovule plus ovary. We have provisionally interpreted them as a dry fruit, more specifically a nutlet. Anatomical studies are underway to try to clarify some of the uncertainties surrounding floral and fruit morphology. This part of the Brassicales clade containing Tiganophytaceae and close relatives provides many opportunities for further studies on vegetative anatomy and comparative genomics.

We were faced with the possibility that the short shoots in *T. karasense* may be the counterparts of the cone-like axillary inflorescences in *Batis*. In *T. karasense* flowers are restricted to the short shoots, and this poses the question whether the latter are not best interpreted as perennial inflorescences (or infructescences), with all their associated leaves representing bracts. Considering the structural homology of stems and leaves with inflorescence axes and bracts, interpretation of these structures may at times be difficult, if not somewhat subjective. Short shoots usually have a specialized function, and although commonly serving as sites for flowering (e.g. Hallé *et al.* 1978, Bell 2008), this does not necessarily make them inflorescences. We have opted for interpreting the axillary side shoots with reduced growth in length as short shoots rather than inflorescences, a decision motivated by the following observations. Shortly after axillary initiation of short shoots on a long shoot, which happens soon after long-shoot development, the associated long-shoot leaves are shed, while those of the short shoots are not, resulting in the short shoots being non-flowering for most of the year and comprising the main photosynthetic units of the plant. The short shoots are long-lived, completely cover the long shoots and show sequential growth flushes of leaves associated with rainfall events, and their stem apices retain the ability to resume growth in length (essentially pleonanthic) to give rise to new long shoots. The vast majority of short shoot leaves never carry a flower in their axil and were thus interpreted as foliage leaves rather than bracts. Between one and four (usually three) flowers emerge from a particular flush of short shoot leaves. Each flower is borne in the axil of a leaf that tends to be slightly (almost imperceptibly so) larger than the sterile leaves, and hence we have, based on this subtle size difference and presence of an axillary flower, referred to the former as bracts. Following the interpretation that the flowers are borne singly and not in an inflorescence, the bulk of a short shoot is made up of sterile foliage leaves, interspersed with a few fertile leaves or bracts. We wish to emphasize that this is an hypothesis, which should be evaluated through developmental study in the future.

Taxonomic treatment

Tiganophytaceae Swanepoel, F.Forest & A.E.vanWyk, *fam. nov.*

Type:—*Tiganophyton* Swanepoel, F.Forest & A.E.van Wyk.

A member of Brassicales, our new family is most closely related to Bataceae and Salvadoraceae and more distantly to Koeberliniaceae. It is morphologically distinct from these and all other known families of the order and easily distinguished by, amongst others, the following combination of characters (for a more comprehensive comparison, see Table 1): dwarf shrub with stems distinctly differentiated into long and short shoots; leaves minute, dimorphic, spirally arranged; glucosinolates present; flowers bisexual, laterally flattened, borne singly in bract axils on short shoots only; calyx, corolla and androecium tetramerous; sepals fused; petals free, not clawed; staminal disc (receptacle?) present; nectary glands absent; ovary deeply bilobed, apparently bilocular; gynophore present, bent in near S-shape; ovary horizontally orientated or occasionally inverted; style gynobasic; ovules two per locule; fruit a dry, persistent, one-seeded nutlet.

Tiganophytaceae differ from Bataceae, Salvadoraceae and Koeberliniaceae by the distinct differentiation of vegetative axes into long and short shoots (*vs.* mainly long shoots), dimorphic foliage leaves (*vs.* monomorphic), fruit a nutlet (*vs.* berry or drupe), ovary bilobed and horizontally orientated or inverted (*vs.* not lobed and upright), and gynobasic style (*vs.* apical). It furthermore is easily distinguished from Bataceae by spirally arranged leaves (*vs.* decussate), hermaphroditic plants (*vs.* monoecious or dioecious), no stipules (*vs.* present, early deciduous), solitary flowers in bract axils (*vs.* in cone-like spikes), sessile petals (*vs.* long clawed in males; absent in females), gynophore present (*vs.* absent), and two ovules per locule (*vs.* one); from Salvadoraceae it differs also by having no stipules (*vs.* present), spirally arranged leaves (*vs.* opposite), solitary flowers in bract axils (*vs.* racemes, panicles or axillary fascicles), free petals (*vs.* basally fused), no nectar glands (*vs.* often present), and well-developed and near S-shaped gynophore (*vs.* absent or short and straight); from Koeberliniaceae it differs also by the lack of thorns (*vs.* present), solitary flowers in bract axils (*vs.* axillary umbel-like racemes), fused sepals (*vs.* free), sessile petals (*vs.* shortly clawed), four stamens (*vs.* eight, rarely ten), no separate nectar glands (*vs.* present), and two ovules per locule (*vs.* many).

As families, Tiganophytaceae, Bataceae and Koeberliniaceae are morphologically distinct. However, our molecular evidence indicates that Bataceae are nested in Salvadoraceae (also Sun *et al.* 2016), making Salvadoraceae paraphyletic, but data on more taxa are needed to fully resolve the possible relationships among these families. A phylogenetic analysis of Brassicales inferred from 72 plastid genes by Edger *et al.* (2018) showed Salvadoraceae and Bataceae to be sisters, but this is of little significance for our use as it was based on a limited sampling of Salvadoraceae, with both *Dobera* and *Azima* absent. From a practical point of view, it would serve little purpose to create heterogeneous families with little or no predictive value, although some may argue that monotypic families do little in the way of summarizing information, perhaps preferring to opt for subfamily or tribal status. Considering available evidence for these families, indications are that the largest one, Salvadoraceae, is heterogeneous as currently circumscribed. For example, *Azima* is, amongst others, morphologically and anatomically distinct from other members of the family (e.g. Takhtajan 2009), and in the past has been placed in a family of its own, namely Azimaceae (Wight & Gardner 1845). However, *Azima* does share some morphological features with *Batis*, notably the possession of unisexual flowers (Ronse De Craene 2005) and an ovary with a false septum, albeit only an apical one (Kshetrapal 1970), as is also present in *Dobera* (Ronse De Craene & Wanntop 2009). Further studies should consider, amongst others, the possible reinstatement of Azimaceae, but alternatively it may be better to accommodate *Azima* in an expanded Bataceae or to subsume Bataceae in an expanded Salvadoraceae.

FIGURE 2. *Tiganophyton karasense*. A. Plant habit and habitat. B. Part of an old long shoot showing short shoots with their rosettes of foliage leaves (mainly) and bracts. C. Young, actively elongating long shoots with short shoots not yet fully developed in leaf axils; arrows indicate where a long shoot emerges from the apex of a short shoot. D. Long shoot densely covered with short shoots, the latter bearing flowers. Photographs: W. Swanepoel.

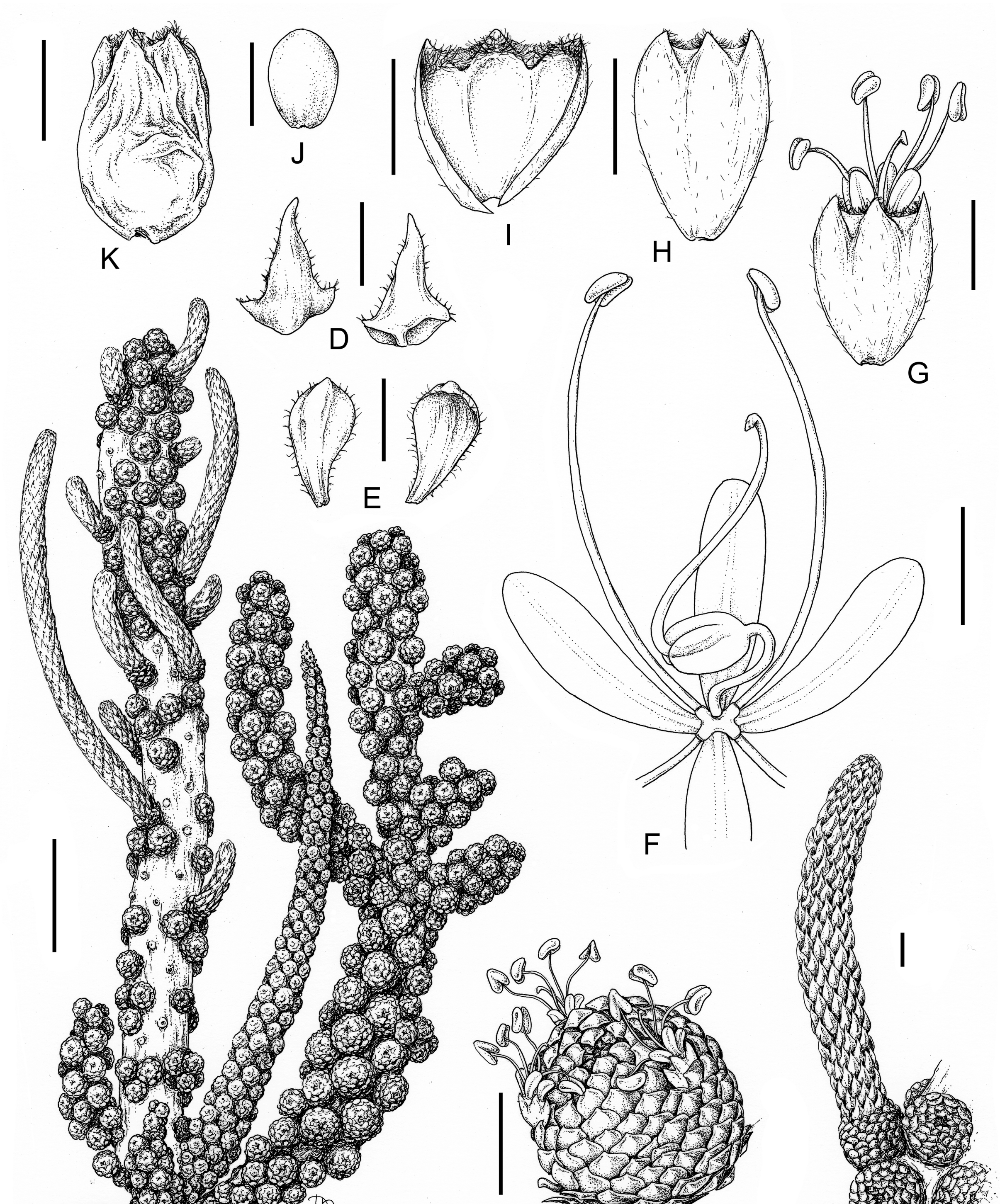

FIGURE 3. *Tiganophyton karasense*. A. Branchlet with short and long shoots; parts of oldest stem with scars left by withered short shoots. B. Short shoot with three flowers. C. Long shoot developing from apex of a short shoot. D. Long shoot leaves; dorsal view left, ventral view right. E. Short shoot leaves; dorsal view left, ventral view right. F. Semi-stylized depiction of a flower (in reality floral parts closely packed with gynophore tightly appressed to ovary), with calyx, two front stamens and a front petal removed. Note gynophore bent in near S-shape, horizontal orientated bilobed ovary, and gynobasic style (portion of style between ovary lobes indicated with stippling; ovary lobes opaque). G. Flower (side view). H. Calyx viewed from outside. I. Calyx opened out and viewed from inside. J. Nutlet. K. Flattened remains of flower with nutlet enclosed in the persistent calyx. Scale bar = 10 mm (A), or 1 mm (B–K). A, C, J & K from *Swanepoel 364* and B & C–I from *Swanepoel 365*. Artist: Daleen Roodt.

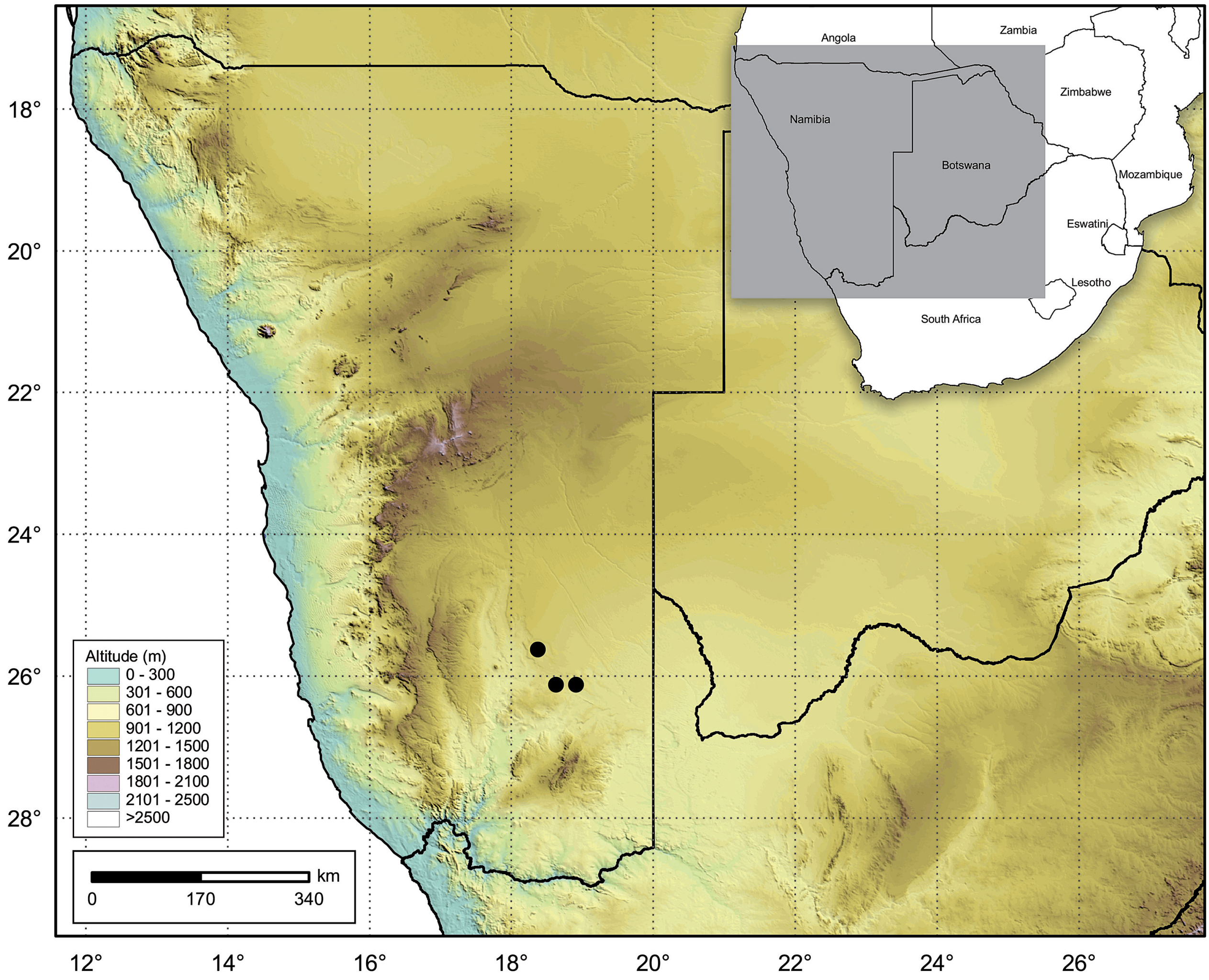

FIGURE 4. Topographical map showing the known distribution (black dots) of *Tiganophyton karasense* in southeastern Namibia. The insert shows a map of southern Africa with names of countries; the grey rectangle indicates the area depicted by the topographical map.

Tiganophyton Swanepoel, F.Forest & A.E.van Wyk, *gen. nov.*

Type:—*Tiganophyton karasense* Swanepoel, F.Forest & A.E.van Wyk.

Description as for the species.

Tiganophyton karasense Swanepoel, F.Forest & A.E.van Wyk, *sp. nov*. (Figs. 2 & 3)

Type:—NAMIBIA. Karas Region: Groot Pan, 38 km northeast of Tses along road, on edge of pan, 2518CB, 1030 m, 19 December 2010, *Swanepoel 365* (holotype WIND; isotypes PRE, PRU).

Evergreen, dwarf shrub, 0.3–0.8 m tall and of nearly equal diam., branching from just below or above ground level, re-branching towards apex; long shoots (dolichoblasts) slender, erect or arcuate-ascending, round, often sulcate, up to 18 mm diam., with long soft hairs, glabrous on older stems, with many short shoots (brachyblasts) in axils of leaves, 3–10 mm long, completely covering shoot, leaves on older short shoots sometimes withering, exposing persistent core as a short thorn/protuberance, often blunt, sometimes only scars remaining; bark stramineous, on older stems ashy with black spots, smooth or longitudinally fissured and peeling. *Leaves* simple, sessile, dimorphic depending on whether on long or short shoots, pale green, often turning yellowish or orange with age, eventually drying to stramineous; on long shoots usually closely spirally arranged, lamina trullate or deltoid, incurved, thickened towards base, bluntly keeled abaxially, flat adaxially, apex acute to attenuate, margin irregularly fimbriate, keel sometimes sparingly fimbriate, ca.

1.2–1.9 × 0.6–0.9 mm; on short shoots spirally arranged, tightly packed in rosettes 2–3 mm diam., 3–10 mm long, lamina ovate, obovate, narrowly obovate or trullate, flat adaxially, slightly incurved especially towards apex, bluntly keeled abaxially, thickened towards acute apex, minutely apiculate, margin often somewhat membranous, fimbriate (less so or fimbrillae lacking on leaves towards base of shoot), 0.8–1.6 × 0.6–1.0 mm, increasing in size towards apex. Inflorescences simple, flowers borne only on short shoot in axils of bracts. Bracts narrowly obovate, oblong-elliptic or subspathulate, concave adaxially, slightly larger than surrounding leaves, otherwise similar. Flowers borne singly, 1–4 per rosette (short shoot) in axil of bracts, laterally compressed, sessile, hypogynous. Calyx tubular, 4-lobed, thin, papery, pale yellow, 1.7–2.1 mm long, lobes 0.8–1.2 × 0.5–0.6 mm, lobes narrowly triangular or narrowly ovate, joined across sinuses between lobes with a thin, membranous tissue (like a "pleat" if viewed from outside), the latter "membrane" with long shaggy hairs on inside towards apex, outside glabrous or sparsely hairy, pale yellow. Disk (receptacle?) 4-lobed. Corolla of 4 petals, free, ± falcate or oblong, prominently longitudinally ridged ("midrib"), sessile, apex obtuse or emarginate, pale white, 2.0–2.3 × 0.5 mm, margins entire or minutely ciliate towards apex. Stamens antesepalous, free, ± equal, spreading, exserted (± 1.6 mm); filaments filiform, slightly flattened, membranous when dry, ± 3.8 mm long, 0.1 mm diam., pale green, inserted on apex of disk lobes with articulation visible, alternating with petals; anthers dorsifixed, introrse, 0.5 mm long; pollen prolate, tricolporate, surface faintly reticulate, lime-yellow. Ovary deeply bilobed, ca. 0.4 mm diam., each lobe with 2 basally attached ovoid ovules, 0.3 × 0.2 mm, glabrous; gynophore slender, 0.7–1.0 mm long, often seemingly (due to lateral flattening of flower) eccentrically inserted on disk, initially erect in young developing flowers, becoming bent in near S-shape following anthesis, resulting in ovary orientated horizontally or occasionally inverted; style ca. 1.8 mm long, elongating to ca. 2.8 mm, tapering from ca. 0.2 mm to 0.1 mm diam., inserted between lobes (gynobasic), stigma capitate, when viewed from above circular, elliptic, trigonous or tetragonal. Fruit 1-seeded, hard, provisionally interpreted as a nutlet (see Discussion), borne in original position of flower within compressed remains of calyx and corolla, 2.1 × 1.6 × 0.8 mm; nutlet globose, ellipsoid or ovoid, slightly compressed, 0.8–1.0 mm diam., smooth, pale yellow-brown.

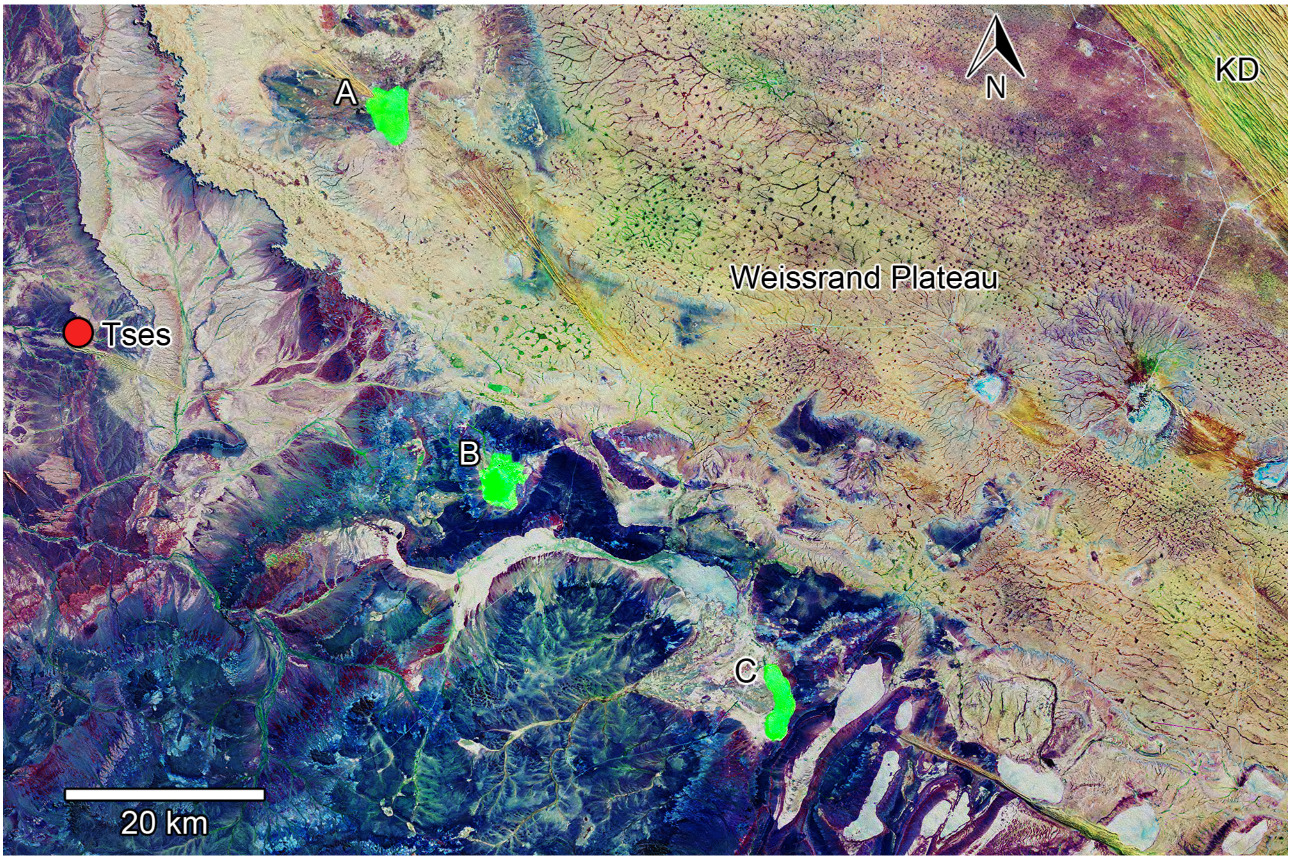

FIGURE 5. Landsat image on which is shown the village of Tses in Namibia and the three seasonal pans (coloured green) from where *Tiganophyton karasense* is known at present: A = Groot Pan; B = Kleinvaalgras Pan; C = Pan at Middelplaas. These pans receive their drainage from the calcrete-covered Weissrand Plateau, the extensive palely coloured area dotted with darkly coloured depressions (dayas). Linear aeolian dunes of the Kalahari (KD) are visible in the upper right-hand corner of the image and believed to have covered the now exposed Weissrand Plateau in the distant past. Image: NASA, based on a tri-decadal global Landsat 7 orthorectified ETM+ Pan-sharpened image.

Phenology:—Flowering during summer (December and January), following good rainfall. The dry fruits (nutlets) are retained amongst the compact leaves and bracts of short shoots, apparently for several seasons.

Distribution and habitat:—At present only known from three localities in arid southeastern Namibia (Figs. 4 & 5), where it is strictly confined to three seasonal pans: Groot Pan, Kleinvaalgras Pan, and a pan on the farm Middelplaas, all within a radius of 35 km. It is rare, although locally common with less than 1000 plants known. *Tiganophyton karasense* grows on recent calcareous substrate in these pans that are underlaid by shales and mudstones of the Prince Albert Formation of the Ecca Group of the Karoo Supergroup. The calcareous nature of the substrate probably has its origin from runoff of the nearby southwestern escarpment of the Weissrand Plateau (Miller & Schalk 1980). Geomorphologically the Weissrand Plateau (Fig. 5) is an area covered by calcrete deposits (calcium carbonate-rich duricrusts) and pockmarked by solution hollows (dayas) associated with aligned drainage channels and old dunes sandwiched between the Nama-Karoo Basin and dune fields of the Kalahari sandveld (Goudie & Viles 2015). Several large, seasonal, endorheic pans are associated with drainage on the Weisrand Plateau itself and especially from its southwestern edge, and these should be explored for possible further populations of the new species (Fig. 5). Plants occur in Karas Dwarf Shrubland, part of the Nama-Karoo Biome, at elevations of 1020–1030 m. Average annual rainfall in the region is 100–150 mm, falling predominantly in summer and autumn, but highly variable and unpredictable (Mendelsohn *et al.* 2002).

The plants were noticed on the edges as well as in the pans, occasionally in shallow water for a couple of weeks following a good rainy season. The species may eventually prove to be more widespread in the area, as what appears to be suitable habitat is not limited to the specific localities where it was found. However, it is absent from several pans in the area with seemingly suitable habitat, but with different underlying geology. Lower rainfall and higher temperatures may have a negative effect on the species since it seems to be more dependent on moisture than the matrix vegetation surrounding the pans and hence its specific habitat requirements. The known range falls within the zone in Namibia with the highest average maximum temperatures during the hottest month (36° C in January/February; Mendelsohn *et al.* 2002).

Conservation status:—*Tiganophyton karasense* is rare and localized with only three locations known. It should be considered as Vulnerable (VU D1) due to its small population size, the latter estimated to number fewer than 1000 mature individuals (IUCN 2012).

Etymology:—*Tiganophyton* is derived from the Greek τηγάνι, *tigani*, a frying pan, referring to the habitat of this species, which can be extremely hot, and φυτών, *fyton*, a plant. The specific epithet "*karasense*" denotes the Karas Region in southern Namibia, where all known localities of the new species are located.

Notes:—The new species has long shoots and short shoots, each with a different type of foliage leaf (Figs. 2B & C, 3D & E). Long shoots develop first, followed by short shoots in the leaf axils. Once the short shoots are well developed, the long shoots lose their leaves. Older long shoots are densely covered by short shoots, the latter appearing as rosettes of tightly packet scale-like leaves and bracts. Flowers are borne on short shoots only (Figs. 2D, 3B) after a rosette has reached a diameter of 2–3 mm. Short shoots seem to continue to grow incrementally for several seasons, and their leaves and bracts are persistent; "annual" whorls of new growth are evident apically. New long shoots develop from the apex of some short shoots (Figs. 2C, 3C), the latter evidently retaining the ability to become long shoots. Nutlets ("seeds") are not actively released from the plants, but remain in their original position hidden amongst the densely packed leaves of the short shoot rosettes. They are probably released only after a plant has died or when a rosette has withered, a process that may take several years under the arid conditions of this habitat.

Additional collections examined (paratypes):—NAMIBIA. Karas Region: Groot Pan, 38 km northeast of Tses along road, near centre of pan, 2518CB, 1030 m, 28 January 2010, *Swanepoel 364* (WIND); Kleinvaalgras Pan, Kleinvaalgras, edge of pan, 2618BA, 1026 m, 12 March 2011, *Swanepoel 367* (WIND); Keetmanshoop, Middelplaas, pan along main road before entrance, 2618BB, 12 February 1998, *Strohbach 3636* (PRE!, WIND!); Middelplaas, along road on edge of pan, 2618BB, 1023 m, 9 January 2011, *Swanepoel 366* (WIND).

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